

Protective Effect of Camel Urine and Milk against Alcohol Induced Liver Damage in Rats

By

AHMED EISA ELHAG IBRAHIM DAFFAALLAH

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Supervisor

Dr. Samia Mohammed Ali El Badwi

*Department of Preventive Medicine and Veterinary Public Health,
Faculty of Veterinary Medicine*

University of Khartoum

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

*قال الله تعالى في سورة الغاشية الآية رقم (17): [أَفَلَا يَنْظُرُونَ إِلَى الْإِبِلِ كَيْفَ خُلِقَتْ].

* وفي سورة الحج الآية رقم (36): [وَالْبُدْنَ جَعَلْنَاهَا لَكُمْ مِنْ شَعَائِرِ اللَّهِ لَكُمْ فِيهَا خَيْرٌ فَاذْكُرُوا اسْمَ اللَّهِ عَلَيْهَا صَوَافَّ فَإِذَا وَجَبَتْ جُنُوبُهَا فَكُلُوا مِنْهَا وَأَطْعِمُوا الْقَانِعَ وَالْمُعْتَرَّ كَذَلِكَ سَخَّرْنَاهَا لَكُمْ لَعَلَّكُمْ تَشْكُرُونَ].

* وفي سورة المؤمنون الآية رقم (21): [وَإِنَّ لَكُمْ فِي الْأَنْعَامِ لَعِبْرَةً نُسْقِيكُمْ مِمَّا فِي بُطُونِهَا وَلَكُمْ فِيهَا مَنَافِعُ كَثِيرَةٌ وَمِنْهَا تَأْكُلُونَ].

* عن أنس رضي الله عنه : {أَنَّ نَاسًا اجْتَنَوْا فِي الْمَدِينَةِ فَأَمَرَهُمُ النَّبِيُّ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ أَنْ يَلْحَقُوا بِرَاعِيهِ يَعْنِي الْإِبِلَ فَيَشْرَبُوا مِنْ أَلْبَانِهَا وَأَبْوَالِهَا فَلَحَقُوا بِرَاعِيهِ فَشَرَبُوا مِنْ أَلْبَانِهَا وَأَبْوَالِهَا حَتَّى صَلَحَتْ أَبْدَانُهُمْ فَقَتَلُوا الرَّاعِيَ وَسَاقُوا الْإِبِلَ فَبَلَغَ النَّبِيُّ صَلَّى اللَّهُ عَلَيْهِ وَسَلَّمَ فَبَعَثَ فِي طَلَبِهِمْ فَجِيءَ بِهِمْ فَقَطَعَ أَيْدِيَهُمْ وَأَرْجُلَهُمْ وَسَمَرَ أَعْيُنَهُمْ}.

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DEDICATION

To the kindest persons on earth who brought me to the life (mother and father) and taught me everything, for their Du'aa to me I'm indebted to dedicate this work to them

To my sweet grand mother

To the soul of my grand parents

To my brothers, sisters, relatives and continually supporting friends

To those who guided me through this life...and taught me a lot of things (Teachers, Professors, and Brothers in ALLAH.....etc...)

To my all colleagues in study and work

To the poor camel owners and suffering patients

To all my lovers I dedicate this modest effort.

Ahmed Eisa Elhag...

With my Regards...

TABLE OF CONTENTS

ITEMS	Page
Title	
Dedication	
Table of Contents	i
List of Tables	v
List of Figures	vi
Acknowledgment	viii
English Abstract	x
Arabic Abstract	xii
Introduction	1
CHAPTER ONE	
LITERATURE REVIEW	3
1.1 Alcohol	3
1.1.1 Acute alcoholism	4
1.1.2 Chronic alcoholism	4
1.2 The liver	4
1.2.1 Liver diseases	6
1.2.2 Liver damage	7
1.2.3 Liver cirrhosis	8
1.2.4 Epidemiology of liver cirrhosis	8
1.2.5 Clinical findings and diagnosis of liver cirrhosis	9
1.2.6 Treatment of liver cirrhosis	9
1.3 Natural treatment of liver diseases	10
1.4 Camels	12

1.4.1 Distribution of Camels	12
1.4.2 Camel milk	12
1.4.3 Camel urine	13
1.4.4 Camel milk and urine as medicine in Sunna	14
1.4.5 Traditional uses of camel's milk and urine	15
1.4.6 Therapeutic uses of camel's milk and urine	16

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials and experimental designs	18
2.1.1 Hepatoprotective effect of one humped camel urine (<i>Camelus dromedaries</i>) in rats	18
2.1.1.1 Animals, housing and management	18
2.1.1.2 Collection and preparation of camel urine	19
2.1.1.3 Administration of camel urine to rats	19
2.1.1.4 Blood samples and parameters	19
2.1.1.5 Hepatoprotective effect of one humped camel milk and urine (<i>Camelus dromedaries</i>) in rats	20
2.1.1.5.1 Animals, housing and management	20
2.1.1.5.2 Collection and preparation of camel milk and urine	21
2.1.1.5.3 Administration of camel milk and urine to rats	21
2.1.1.5.4 Blood samples and parameters	21
2.2 Methods	22
2.2.1 Chemical methods used for determination of serum constituents	22
2.2.1.1 Aspartate amino transferase (AST) (SGOT)	22
2.2.1.2 Alanine amino transferase (ALT) (SGPT)	23

2.2.1.3 Alkaline phosphatase (ALP)	24
2.2.1.4 Total protein	24
2.2.1.5 Albumin	25
2.2.1.6 Billirubin	25
2.2.2 Haematological methods	26
2.2.2.1 Packed cell volume (PCV)	26
2.2.2.2 Haemoglobin (Hb) concentration	26
2.2.2.3 Total Red blood cells (RBCs) count	27
2.2.2.4 Mean corpuscular volume (MCV)	27
2.2.2.5 Mean corpuscular haemoglobin (MCH)	27
2.2.2.6 Mean corpuscular haemoglobin concentration (MCHC)	28
2.2.3 Histopathological methods	28
22.4 Statistical analysis	28

CHAPTER THREE

RESULTS

3.1 Effect of camel urine against ethanol induced liver damage in rats	29
3.1.1 Clinical signs	29
3.1.2 Post-mortem findings	29
3.1.3 Changes in serum enzymes	29
3.1.4 Changes in serum metabolites parameters	33
3.1.5 Changes in haematological parameters	33
3.1.6 Changes in red blood cell indices values	38
3.1.7 Histopathological changes	38
3.2 Effect of camel milk and urine against ethanol induced liver damage in rats	41

3.2.1 Clinical signs	41
3.2.2. Post-mortem findings	41
3.2.3 Changes in serum enzymes	41
3.2.4 Changes in serum metabolites parameters	45
3.2.5 Determination of haematological parameters	45
3.2.6 Calculation of red blood cell indices	45
3.2.7 Histopathological changes	51

CHAPTER FOUR

DISCUSSION

4.1 Effect of camel urine against ethanol induced liver damage in rats	55
4.2 Effect of camel milk and urine against ethanol induced liver damage in rats	56

CONCLUSION AND RECOMMENDATIONS

REFERENCES

LIST OF TABLES

Table No.	Subject	Page
1	Changes in serum enzymes constituents of rats treated with camel urine and ethanol	30
2	Changes in serum metabolites concentrations of rats treated with camel urine and ethanol	34
3	Changes in haematological parameters in rats treated with camel milk urine and ethanol	37
4	Changes in values of red blood cell indices in rats treated with camel urine and ethanol	39
5	Changes in serum enzymes constitutes of rats treated with mixture of camel milk and urine plus ethanol	42
6	Changes in serum metabolites concentrations of rats treated with mixture of camel milk and urine plus ethanol	46
7	Changes in haematological parameters in rats treated with mixture of camel milk and urine plus ethanol	49
8	Changes in values of red blood cell indices in rats treated with mixture of camel milk and urine plus ethanol	50

LIST OF FIGURES

Figure No.	Subject	Page
1	Changes in serum AST levels of rats treated with camel urine and ethanol	31
2	Changes in serum ALT levels of rats treated with camel urine and ethanol	31
3	Changes in serum ALP levels of rats treated with camel urine and ethanol	32
4	Changes in total protein concentration in rats treated with camel urine and ethanol	35
5	Changes in albumin concentration in rats treated with camel urine and ethanol	35
6	Changes in billirubin concentration in rats treated with camel urine and ethanol	36
7	Histopathological changes in livers of rats treated with ethanol, camel urine and Silymarin	40
8	Changes in serum AST levels of rats treated with mixture of camel milk and urine plus ethanol	43
9	Changes in serum ALT levels of rats treated with mixture of camel milk and urine plus ethanol	43
10	Changes in serum ALP levels of rats treated with mixture of camel milk and urine plus ethanol	44
11	Changes in total protein concentration in rats treated with mixture of camel milk and urine plus ethanol	47

12	Changes in albumin concentration in rats treated with mixture of camel milk and urine plus ethanol	47
13	Changes in billirubin concentration in rats treated with mixture of camel milk and urine plus ethanol	48
14	Histopathological changes in livers of rats treated with ethanol, mixture of camel milk and urine and Silymarin	52

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ABSTRACT

This study was designed to investigate the protective effect of camel urine alone and camel milk mixed with camel urine against alcohol induced liver damage in rats. The study was conducted at the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum. It was performed on fifty Wistar Albino rats of both sexes weighing 85-105 gm, and was continued for four weeks (28 days). On day (29) the rats were slaughtered. Liver damage was induced by oral administration of 10% Ethanol at 5g/kg body weight.

The first experiment was designed to evaluate the effect of camel urine alone against ethanol induced liver damage. Twenty five rats were used, divided into 5 groups. Rats in group 1 served as control and received normal saline orally, rats in group 2 were given orally with ethanol 10%, and rats in group 3 received orally Silymarin (standard drug) (50 mg/kg body weight) and after 3 hours the same group received ethanol 10%. Group 4 rats received camel urine at 1ml/100gm body weight by an oral intubation and rats of group 5 were administered orally with camel urine at 1ml/100gm body weight and after 3 hours received ethanol 10%. The experiment continued for 28 days, and on day 29 the rats were slaughtered.

The second experiment was conducted to evaluate the effect of the mixture of camel milk and urine against ethanol induced liver damage. Twenty five rats were used and divided into 5 groups. The rats of groups 1, 2 and 3 represented control, ethanol and Silymarin respectively, by the same schedule as the first experiment. The rats in group 4 received a mixture of camel milk and urine (1:1) at the rate of 2ml/100gm body weight, and group

5 were administered with the mixture of camel milk and urine by the same dose as in group 4 and then after 3 hours received ethanol 10% (5g/kg body weight). The experimental continued for 28 days.

Oral administration of camel urine, three hours before alcohol (10% ethanol), to the rats in the first experiment reduced levels of aspartate aminotransferases (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), induced by alcohol intoxication, beside noticeable stability and decrease in serum metabolites (total proteins, albumin and billirubin) concentrations which increased in alcoholic intoxicated rats. These results are better than the results obtained in the reference drug (Silymarin) group. The results of the serum enzymes and metabolites were correlated with histopathological changes.

On the other hand, administration of camel milk mixed with the camel urine to rats in group 5 of the second experiment which were treated with alcohol after three hours, resulted in significant reduction of the levels of liver serum enzymes (AST, ALT, and ALP) and in stabilization and reduction of concentration of the serum metabolites (total protein, albumin and billirubin) compared with Silymarin + ethanol group, even more strong than using the camel urine alone. These results were verified by the histopathological changes.

In Conclusion, the camel urine alone can act as protective agent against liver damage, but the camel milk mixed with camel urine has high protective effects and that could be attributed to the antioxidant activity or, possibly, to the chelating effects of camel milk and urine on toxicants specially alcohol.

المستخلص

هذه الدراسة أجريت لتقصي التأثير الوقائي لبول الإبل منفرداً وبعد خلطه بلبن الإبل ضد تلف الكبد في الجرذان المسبب بواسطة الكحول. أجريت الدراسة في كلية الطب البيطري بجامعة الخرطوم علي خمسين من جرذان الويستر البيضاء من كلا الجنسين بأوزان تتراوح بين 85 و 105 جم، ولمدة أربعة أسابيع (28 يوماً) وفي اليوم التاسع والعشرين ذبحت الجرذان. أحدث تلف الكبد بتجريع 10% من الإيثانول بمعدل 5 جم لكل كجم وزن حي.

صممت التجربة الأولى لتقييم تأثير بول الإبل منفرداً ضد تلف الكبد في الجرذان المسبب بواسطة الكحول. أستخدم 25 جرذاً، قسمت إلى 5 مجموعات. الفئران في المجموعة 1، استخدمت كشاهد وتلقت محلول ملحي طبيعي، جرذان المجموعة 2 أعطيت 10% إيثانول (كحول) عن طريق الفم والجرذان في المجموعة 3 تلقت فموياً دواء السيليمارين القياسي بمعدل 50 ملجم / كجم وزن حي وبعد 3 ساعات أعطيت المجموعة نفسها 10% إيثانول. أما المجموعة 4 فأعطيت بول الإبل بمعدل 1 مل لكل 100 جم من وزن الجسم عن طريق التجريع بالفم، والمجموعة 5 أعطيت فموياً بول الإبل بمعدل 1 مل لكل 100 جم من وزن الجسم وبعد 3 ساعات تم إعطائها 10% إيثانول. هذه التجربة استمرت لمدة 28 يوماً وفي اليوم 29 ذبحت الجرذان.

أجريت التجربة الثانية لتقييم تأثير خليط لبن وبول الإبل ضد تلف الكبد في الجرذان المسبب بواسطة الكحول. أستخدم 25 جرذاً، قسمت إلى 5 مجموعات. الفئران في المجموعات 1، 2 و 3 مثلت معاملات الشاهد و الإيثانول والسيليمارين علي التوالي، بنفس الترتيب الجدولي الموضوع في التجربة الأولى وأعطيت الفئران في المجموعة 4 خليط لبن و بول الإبل بنسبة 1:1 وبمعدل 2 مل لكل 100 جم من وزن الجسم عن طريق التجريع بالفم، والمجموعة 5 أعطيت فموياً خليط لبن وبول الإبل بنفس جرعة المجموعة 4 وبعد 3 ساعات تم إعطائها 10% إيثانول. استمرت التجربة كانت 28 يوماً.

إعطاء بول الإبل عن طريق الفم قبل ثلاث ساعات من إعطاء الكحول (الإيثانول 10%) للجرذان في التجربة الأولى أدى إلي تقليل مستويات إنزيمات الكبد المصلية AST و ALT و ALP

والتي نتجت عن التسمم بالكحول، بجانب الإستقرار والإنخفاض الملحوظ في تراكيز المستقلبات المصلية (البروتين الكلي والألبومين والبيليروبين) والتي إرتفعت في الفئران المسممة بواسطة الكحول. هذه النتائج كانت أفضل من النتائج المتحصلة من الدواء المرجعي (سيليمارين)، وإرتبطت نتائج الإنزيمات والمستقلبات المصلية مع التغيرات النسيجية المرضية.

من جهة أخرى، أسفر إعطاء لبن الإبل مختلطاً مع بولها للفئران في المجموعة الخامسة للتجربة الثانية، والتي تم إعطائها الكحول بعد ثلاث ساعات، أسفر عن إنخفاض معنوي في مستويات إنزيمات الكبد المصلية (AST)، (ALT) و (ALP) وعن إستقرار وتدني في تراكيز مستويات المستقلبات المصلية (البروتين الكلي والألبومين والبيليروبين) مقارنة مع مجموعة السيليمارين + الإيثانول، حتى أنها كانت أكثر فاعلية من استخدام بول الإبل منفرداً". هذه النتائج كانت أيضاً مرتبطة بالتغيرات النسيجية المرضية.

تشير نتائج الدراسة إلي أن بول الإبل يمكن أن يمثل عاملاً وقائياً ضد تلف الكبد، كما أنها تثبت أن لبن الإبل وبولها مختلطين معاً" لهما تأثيرات وقائية أقوى ضد تلف الكبد الذي يسببه الكحول ، ويمكن أن يعزى ذلك إلى النشاطات المضادة للأكسدة أو ربما يكون ناتجاً عن الآثار المخيلية لبن الإبل وبولها علي المواد السامة خصوصاً الكحول.

INTRODUCTION

Liver is the first organ to metabolize all foreign compounds and hence it's susceptible to many different diseases (Sakar *et al*, 2005). Alcohol administration is one of the most common causes of chronic liver diseases in the world and it was found that, alcohol affects the liver not only through nutritional disturbances, but also through direct toxicity, because it is predominant metabolism in the liver is associated with oxidation, reduction changes and oxidative stress (Lieber, 2004). The body's natural defense against free radicals such as antioxidants are inhibited by alcohol consumption resulting in the liver damage (Augustyniak *et al.*, 2005).

Liver diseases are a major worldwide health problem, with high endemicity in developing countries. They are mainly caused by chemicals and some drugs when taken in very high doses. Despite advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or helps to regenerate hepatic cells. There is urgent need, therefore, for effective drugs to replace and supplement those in current use (Adewusi and Afolayan, 2010).

Camel milk and urine has been claimed to cure or benefit patients with diabetes, tuberculosis, stomach ulcers, gastroenteritis, cancer, allergies, infections, parasites, autism and even AIDS. Camel milk has been widely used in a number of countries as food additives and for curing some commonly occurring diseases, among other properties, camel's milk is high in vitamin C, low in vitamin A, and low in fat compared to cow's milk, and it is tolerated by those who are lactose intolerant (Kamal *et al.*, 2007; Al-Hashem, 2009). It is different from

cow's milk in many other ways, but the clinical significance of those differences is not clear; recently camel milk and urine has been deeply studied for special properties.

In India camel milk is used therapeutically to treat dropsy, jaundice, problems of spleen, tuberculosis, asthma, anaemia, piles and diabetes (Rao, *et al* 1970). Camel milk is used in cases of haemorrhoids (piles), and also used for improvement of bone formation (Yagil, 1982), meanwhile, Haddad (2006), reported that, the camel's milk has been used to cure diseases caused by chronic imbalance of the liver, such as jaundice, oedema, and swelling of the belly, and it has been shown throughout the history of medical science till today that urine has a profound medical use such as effectiveness against allergies, skin conditions, fever, burns, tuberculosis and fertility (Natalie, 2002). It is also reported by Ohaj, 1998 that, camel urine is useful in treatment of ascites.

There are also a few studies suggesting a possible benefit in allergies (Al-Haj and Al-Kanhal, 2010), in peptic ulcers (Sharmanov *et al.*, 1981), in infections such as hepatitis (Sharmanov *et al.*, 1982), and in Schistosomiasis (Maghraby *et al.*, 2005).

*** The objectives of this study are to:**

- 1- Evaluate the protective effect of camel milk and urine on alcohol induced hepatotoxicity.
- 2- Compare the protective effect of camel milk and urine together with camel urine alone against alcohol induced hepatotoxicity.

CHAPTER ONE

LITERATURE REVIEW

1.1 Alcohol

Alcohol (ethanol) is number one favorite mood altering drug in the United States (Lieber, 1991), it is not only a psychoactive drug but is also considered as part of the basic food supply in many societies, after ingestion most of alcohol is metabolized in the liver by hepatic alcohol dehydrogenase enzyme. A large intake of alcohol has enormous effects on nutritional status thereafter absorption, destruction and elimination begin, the kidneys and lungs excrete about one-tenth of the total alcohol ingested unchanged, the remaining alcohol undergoes oxidation. The oxidation of alcohol produces energy that results in temporary reduction of fear and conflict thereby strengthening drinking desire (Charness *et al.*, 1989).

Alcohol has been a constant presence in african social life for centuries as it has been in most parts of the world (Obot, 2006), alcohol social consequences affect individuals other than the drinkers, such as traffic casualties, or incidence of violence in the family (WHO, 2004). Aragi is the native alcoholic drink commonly used in the Sudan; it has been the drink of choice to most people who take alcohol, due to its affordable price and availability. Both the amount of the drink consumed and the number of people taking it are assumed to have risen. This implies an increase in the risk of the emergence of toxicity due to Aragi intake (ElGamal *et al.*, 2003), although Aragi consumption is widely spreading in Sudan, research in the adverse effects of Aragi is scarce, sparse and scanty.

1.1.1 Acute Alcoholism

Alcoholism is the most widely used term to describe patients with alcohol problems (Goldman and Ausiello., 2004). The major effect of acute alcoholism is associated with the central nervous system (Kathryn *et al.*, 1993). In general ethanol toxicity, with its many complications at a time, affects different organs causing several disorders. The multiple pathogenicity of alcoholism demands multiple modes of therapeutic approach to fight against such problems by modulating enzyme activities, metabolism, receptor functioning, signal transduction machinery and scavenging free radicals at various levels (Xu *et al.*, 2005).

1.1.2 Chronic Alcoholism

Chronic alcohol administration results in increased levels of endotoxin in the portal circulation, thereby activating Kupffer cells to produce toxic mediators that cause liver injury (Nanji *et al.*, 1993; Thurman, 1998). Chronic alcohol consumption increases Nitric Oxide level and may lead to toxicity by peroxynitrite, a potent oxidant (Venkatraman *et al.*, 2004). Overproduction of reactive nitrogen species and reactive oxygen species (RNS/ROS) may occur when its generation in a system exceeds the system's ability to neutralize and eliminate them.

In addition, both acute and chronic ethanol administration leads to formation of cytokines, especially TNF-alpha by hepatic Kupffer cells, which have a significant role in liver injury (Zakhari and Li, 2007).

1.2 The Liver

The liver is the largest gland and the most important organ in the body that performs several vital functions that essential for the life and it has enormous functional reserve and many regulatory and storage

functions, also plays a vital role in regulating various physiological processes, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. The liver removes excess amino acids from the blood, converting them to urea, which is excreted by the kidneys (Shanani, 1999; Subramoniam and Pushpangadan, 1999). It helps in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction. The liver receives the products of digestion, converts glucose to glycogen (a long chain carbohydrate used for storage and breakdown of fats). In addition, it aids metabolism of carbohydrate, protein and fat, detoxification, secretion of bile and storage of vitamins (Ahsan *et al.*, 2009). The liver also produces bile and blood-clotting factors, and eliminates damaged red cells and toxins such as Alcohol from the blood. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds, culminating in liver dysfunction (Bodakhe and Ram, 2007).

The liver is a vital organ of paramount importance involved in the maintenance of metabolic functions and detoxification from the exogenous and endogenous challenges like xenobiotics, drugs, viral infections and chronic alcoholism (Ramachandra *et al.*, 2007). An exposure to the above mentioned challenges shows that the natural protective mechanism of the liver are over powered and leads to hepatic injury (Wolf, 1999).

For the importance of the liver and its disease susceptibility there are about (600) commercial therapeutic preparations against hepatitis alone (Ortomans, 1979).

1.2.1 Liver Diseases

Liver diseases are one of the common diseases around the world. It has many types and caused by different reasons, and is characterized by specific symptoms. Liver diseases remain one of the major threats to public health and are a worldwide problem (Asha and Pushpangadan, 1998). The causes of liver diseases or damage may be:

Toxical, Viral, Bacterial, Protozoal, Fungal, Parasitic, Nutritional, Chemical, other infections and autoimmune disorders.

Chemicals causes is like acetaminophen (in large doses), excess consumption of alcohol. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Dianzani *et al.*, 1991). While alcohol is one of the main causes of end-stage liver disease worldwide, alcoholic liver disease is the second most common reason for liver transplantation in the United States (Mandayam *et al.*, 2004). Due to increased frequency of drinking and change of diet construction, such as the increase of fat content, the incidence of liver diseases has increased in China, becoming another important risk factor for morbidity and mortality in addition to viral hepatitis (Zhuang and Zhang, 2003). The spectrum of alcoholic liver disease ranges from fatty liver to alcoholic hepatitis and ultimately fibrosis and cirrhosis (Tuma and Sorrell, 2004).

Liver disorders still remain common and unconquered. Modern medicines have little to offer for alleviation of hepatic diseases and only limited numbers of drugs are available for the treatment of liver disorders.

1.2.2 Liver damage

Liver is an important organ for detoxification and metabolism, and it has a good repairing capability. Common damage caused by non-persistent or mild toxicity can be repaired through certain mechanisms.

Hepatic damage is always associated with the cellular necrosis, the increase in tissue lipid peroxidation and the depletion in the tissue glutathione (GSH) levels (Sandy and Ben, 1998). Moreover, serum levels of many biochemical markers like aspartate transaminase (AST), alanine transaminase (ALT), serum alkaline phosphatase (ALP), triglycerides, cholesterol and bilirubin are also elevated. However, liver damage induced by persistent alcohol overdose or virus attack may cause chronic hepatitis, cirrhosis, and even hepatoma lead to death. In recent years, the death rate of male hepatoma is the highest among the ten major causes of death by cancer in Taiwan (Department of Health, Taiwan, 1993). Reports have described that liver damage caused by alcohol increased with the dosage, and the severity of damage is different among gender, species, and genes. For example, about 50% of Orientals lack the important enzyme for alcohol metabolism, aldehyde dehydrogenase. Therefore, their tolerance of alcohol is poor and liver damage occurs easily (Lieber, 1997; 1998), (Brody, 1999).

Enhanced oxidative stress, decreased antioxidant status and nitrosative stress appear to be chiefly responsible for alcoholic liver damage (Lu and Cederbaum, 2008). The close relation between ethanol and liver damage is mainly due to the fact that 80% of ingested alcohol is metabolized in the liver. During ethanol metabolism many reactive oxygen species are generated via cytochrome P450 2E1 (Tuma and Casey, 2003). Nitric oxide (NO) is an important mediator of many

physiological functions, and its role in the pathogenesis of many diseases is gaining recognition (Pacher *et al.*, 2007).

The main sign of liver damage is jaundice, which is a clinical sign and often arises in diseases of liver and billiary system, but also in diseases in which there are no lesion of these organs. It is result of accumulation of billirubin. The staining is more prounced with direct billirubin than indirect billirubin.

1.2.3 Liver cirrhosis

Liver cirrhosis is a morphologic alteration of the liver that has received a great amount of alteration. Histologically cirrhosis is characterized by presence of separe of collagen distributed throughout the liver cells (Gabriel, 1986). Cirrhosis is associate with alcohol abuse, (Cotran. *et al* 1989; Hunt and Mccosker 1992). Dietary derangement can induce fatty change in the liver. Billiary cirrhosis following chronic obstruction of bile flow (Cholestasis). A greater exposure to hepatotoxic drugs and chemicals cause diffuse liver toxicity leads to cirrhosis (Wayne, 1996). In the later stage of cirrhosis many complications may develop, such as ascitis, gastrointestinal bleeding, and mental deterioration encephalopathy (Sax, *et al.*, 1980; Fracer and Ariell, 1985). Hepatocellular carcinoma develops in as many as 10% of human with long standing cirrhosis.

1.2.4 Epidemiology of liver cirrhosis.

Cirrhosis is a leading cause of death in the United States (Anon, 1983). Worldwide the annual death rate from cirrhosis of all causes is as high as 15 to 40 persons per 100,000 populations, (WHO, 1985). In the third world countries, children are frequently affected following maternally acquired hepatitis (Cotran, *et al.*, 1989; Anon, 1983). In 1155

patients with cirrhosis from a variety of causes, the overall 5 year survival was about 40% (D'Amico *et al.*, 1986) the cause of death was liver failure in 49%, hepatocellular carcinoma in 22%, bleeding in 14%, hepatic renal syndrome in 8%, and other causes in the remainder. Cirrhosis has become one of the 5 most frequent causes of death in persons over the age of 40 years (Anon, 1983).

1.2.5 Clinical findings and diagnosis of liver cirrhosis

Cirrhosis is insidious in its development and often produces no clinical manifestations. Up to 50% of all cases discovered, only at the time of post mortem examination. Many patients seek medical help complaining of vague, non-specific symptoms such as weight loss, loss of appetite, nausea, vomiting, and ill-defined digestive disturbances. Others were acutely ill with the full syndrome of acute alcoholic hepatitis (Precursor to cirrhosis), they have jaundice, mildly elevated serum aminotransferases (ALT and AST), and alkaline phosphates levels, a low serum albumin level, evidence of impaired coagulation (Prolonged prothrombin time) and might be equidistant pain. Despite extensive investigation of liver function and pathologies, there is no effective therapy for many liver diseases. At base only symptomatic management (rather support or promote the process of healing or regeneration of the liver). Jaundice and ascetic are signs of advance liver damage and are late signs of cirrhosis (Chrestopher, 1995).

1.2.6 Treatment of liver cirrhosis

The drugs available in the modern system of medicine are the corticosteroids and/or immunosuppressive agents, which bring about only symptomatic relief (Handa, *et al.* 1986). Sudanese traditional camel owners used camel urine with or without milk for the treatment of

jaundice, hepatomegaly, splenomegaly, ascites, and many internal disorders. Management of cirrhosis is largely symptomatic.

Some of the interesting drugs that used for liver cirrhosis are Thiamine, Vitamin K, Spironolactone, Sodium tetradecyl sulfate or ethanolamine oleate, Vasopressin, Dopamine.

1.3 Natural treatment of liver diseases

The health is equilibrium between the mind, the body, and the external world (environment), where disease is a disruption of this harmony. Prevention and treatment involve creating the condition in which the body could maintain and cure itself through its internal healing mechanism. When disease did manifest itself-specific intervention would be applied, but natural cures such as dietary changes were preferred over drugs so people generally treated their illness with prayer and common sense approaches as good food, rest and whatever substances they found in nature that were traditionally known to have medical qualities such as simple herbs, plants, minerals, urine etc. (Zaki *et al.*, 1984).

Acute and chronic liver diseases constitute a global concern and medical treatments for these diseases are often difficult to handle and have limited efficiency (Lee *et al.*, 2007). Therefore, there has been a great deal of interest in the role of complementary and alternative medicines for the treatment of various liver diseases. In spite of the tremendous advances in modern medicine, there is no effective drug available that stimulates liver function, offer protection to the liver from damage or help to regenerate hepatic cells (Chattopadhyay, 2003). Developing therapeutically effective agents from natural products may reduce the risk of toxicity when the drug is used clinically. It is therefore

necessary to search for alternative drugs for the treatment of liver diseases to replace currently used drugs of doubtful efficacy and safety.

A number of drugs derived from plants have been reported to have hepato protective activity and are using in treating many liver disorders such as different extracts of *Apium graveolens* and *Croton oblongifolius* which were tested for their hepato protective activity against CCL4 induced hepato toxicity in albino rats. The methanolic extracts showed the most significant hepato protective activity compared with the standard drug Silymarin (Ahmed *et al.*, 2002). Other extracts of petroleum ether and acetone also exhibited a potent activity.

Upadhyay *et al.*, (2000) found that the ethanolic extracts of *Cassia tora* and its butanol fraction and hepato protective activities in rats. Whereas the benzene fraction was found to be inactive.

In Turkish ethanolic extract of seven plants were used as hepato protection, these plants are; *Carduns acanthoides*, *C. nutans*, *Cichorium intybus*, *Fumaria aseplae*, *F. vailantii*, *Gentiana olivieri* and *Platago lanceolatl* (Aktay *et al.*, 2000).

In Jordian folk medicine hepato protective effect of boiled and non-boiled aqueous extracts of *Pistacia lentiscus*, *Phyllyrea latifolia* and *Nioctiana glauca* showed their effectiveness in the treatment of jaundice which evaluated in vivo using CCL4-intoxicated rats as an experimental model- plants extracts administered orally at dose of 4 ml/kg body weight. The effect of boiled extract was more pronounced than that of the non-boiled extract (Janakat and Al-Merie, 2002). Also significant hepato protective effect was obtained against CCL4 exhibited potent activity.

1.4 Camels

1.4.1 Distribution of Camels

Camels (*Camelus dromedarius*) are important as dairy animals in certain regions of the world. The total population of camels in the world is about 18 million (Al-Hadrami, 2003). The camels were domesticated particularly in arid and semiarid zones of North and East Africa, the Indian subcontinent, and Saudi Arabia. Dromedaries are mainly used for milk production, whereas the bactrians, are more prevalent in desert and semi desert areas of northwestern Asia.(Mehaia *et al.*,1995).

The camel (*Camelus dromedarius*) is of significant socio-economic importance in many arid and semi-arid parts of the world and its milk considered as important component of human diets in these regions. It is widely admitted that dromedary camels produce more milk of high nutritional quality and can stay for long period out of refrigerator in environment that may be rightly termed as hostile in terms of extreme temperature, drought and lack of pasture (Bakht and Arshad, 2001).

1.4.2 Camel Milk

Milk is the main source of nutrition for the neonates, and provides all the essential nutrients for growth and development, it contains proteins, minerals, carbohydrates, fatty acids, growth factors and immune modulators, (Halima *et al.*, 2006).

Camel's milk is different from other ruminant milk; having low cholesterol, low sugar, high minerals (sodium, potassium, iron, copper, zinc and magnesium), high vitamin C, B2, A and E, low level of protein, also contains many of nutrients like fat, protein, lactose, vitamins, minerals, and Immunoglobulin (Knoess, 1979). It has no allergic properties and it can be consumed by lactase deficient persons and those

with weak immune systems, most of the camel's milk is used fresh or when it has just turned sour (Farah and Bachman, 1989). Yagil *et al.*, (1994), reported that, the milk production reach amounts up to 20 liters a day or more. Camel's milk is richer in immunoglobulins than human milk. However, its contents of lactoferrin and lysozyme were very low, camel's milk and colostrums were shown to be rich in protective proteins, especially IgG2 and IgG3, which revealed to be a potential source of inhibitory antibodies (Halima *et al.*, 2006).

The milk of camel can be mixed with camel urine and used by ladies for treatment of hairs, stomach pain, gum and teeth pain, eyes affections, skin injuries and infections, snake bite, camel mange, liver tumors, prevention of thirst and hunger and to wake up drunk man (Baesmel, 2004).

1.4.3 Camel urine

The camel excretes about 2-4 liters of urine per day and these decreases with dehydration to about 0.5 litre of highly concentrated urine, especially with sodium and potassium salts, (Schmidt-Nelsen, 1964). Urinary Na drops significantly after three days of food deprivation (Dahloborn *et al.*, 1992). The values of Cl in hydrated camel in literature vary greatly, 492-902 Mequ/L (Wilson 1984); 332-429 Mequ/L (Manifield and Tinson, 1996).

Chemical constituents of camel urine were investigated to understand how withstanding prolonged dehydration periods. The excretion of highly concentrated urine with recycling of up to 97% of urea without affecting the nitrogen balance (Mousa *et al.*, 1983) and low uric acid levels (Mura *et al.*, 1986) were among the major features of camel urine in this respect. Recently increasing interest in biological activity studies of camel urine, particularly in the Middle East revealed

that it contains, high potassium, urea, and creatinine and low sodium and uric acid levels (O'haj *et al.*, 2000). In contrast, however, low levels of urea and creatinine and considerably high sulphates and uric acid were reported for Saudi camels (Amer and Alhendi, 1996). Vitamin C was found to be higher in female Sudanese camel breeds than the males (Mohamed and Beynen, 2002).

1.4.4 Camel milk and urine as medicine in Sunna

In Sunni Islam, the Sahih Bukhari, which forms one of the six major Hadith collections quotes the Prophet Muhammad advocating drinking camel's urine as a medicine in several verses.

Sahih Bukhari volume 7, book 71, number 590:

Narrated Anas: The climate of Medina did not suit some people, so the Prophet ordered them to follow his shepherd, i.e. his camels, and drink their milk and urine (as a medicine).

Sahih Bukhari volume 8, book 82, number 794:

Narrated Anas: Some people from the tribe of 'Ukl came to the Prophet and embraced Islam. The climate of Medina did not suit them, so the Prophet ordered them to go to the (herd of milch) camels of charity and to drink, their milk and urine (as a medicine). They did so, and after they had recovered from their ailment (became healthy) they turned renegades (reverted from Islam) and killed the shepherd of the camels and took the camels away. The Prophet sent (some people) in their pursuit and so they were (caught and) brought, and the Prophets ordered that their hands and legs should be cut off and that their eyes should be branded with heated pieces of iron, and that their cut hands and legs should not be cauterized, till they die. Al-Bukhari (2009).

Although it is recorded in the Bukhari that Prophet Muhammad

advocated drinking camel urine as a medicine to his followers, and did not describe it as dirty or demeaning, later commentators find urine to be something that is "filth in an extreme degree" without denouncing its alleged medicinal properties. Abu Hanifa said that it's disliked, but not forbidden, to drink the urine from camels. Abu Yusuf said that urine from camels can be consumed for medicinal purposes.

1.4.5 Traditional uses of Camel's milk and urine

Beside milk, meat and wool, camel urine has attracted special significance in terms of folk medicine for people in the deserts (Al-Awadi *et al.*, 2004). The folklore of many camel rearing societies is rich of stories and practices describing the medicinal properties of camel's milk and its urine (Yagil 1982). Historically the use of camel milk and urine goes back to the era before Islam, strengthened by the Prophetic prescription to sick Bedouins and widely practiced by medieval Arab physicians (O'haj *et al.*, 2000) and documented in Chinese medical encyclopedia since the sixteenth century. A wide range of diseases is believed by Bedouins to be treated by consumption of raw or fermented camel milk. These include: infectious diseases like tuberculosis (Faye, 2005), chronic digestive ailments like: peptic ulcer (Sharmanov *et al.*, 1981), diabetes mellitus (Agrawal *et al.*, 2007) and hepatitis (Sharmanov *et al.*, 1982). Fermented camel milk products were reported to possess antihypertensive action in addition to Samna, a Sudanese and Egyptian traditionally concentrated milk fat product similar to ghee (Abou-Donia *et al.*, 2003), which is used as a cosmetic and wounds healing.

The traditional use of human and animals urine goes back to the early Jewish periods in the 1st to the 5th centuries (CE) (Lev 2006). Ample information is available on the traditional uses and studies of human urine. Camel urine was used for centuries in Arab countries both

by ingestion and topical applications. Historically, it is known to be used by Arab before Islam (Ali, 1993).

1.4.6 Therapeutic uses of Camel's milk and urine

Literature overview shows that most of the studies on camel milk and urine show that it is a high quality drink and since ancient times people have this product for curing a number of diseases (Shabo *et al.*, 2005; Redwan and Tabll, 2007).

Nowadays the therapeutic uses of camel's milk are considered as a new revolution in most countries. The Camel's milk contains many useful components the relatively high concentrations of vitamin C in Camel's milk, aids in the improved liver function (Yagil *et al.*, 1994). Shamsia (2009), reported that, certain concentrations of antimicrobial factors in Camel and human milks. A series of metabolic and autoimmune diseases are successfully being treated with camel's milk, such as beneficial role of raw camel's milk in chronic pulmonary tuberculosis which has been observed (Mal *et al.*, 2001). In repeated trials, it was observed that there was 30-35% reduction in daily doses of insulin in patients of type 1 diabetes receiving raw camel's milk (Agrawal *et al.*, 2002).

The medicinal properties of the Arabian camel were known to Arab physicians. In his 'Magisterial Canon'. Ibn Sina (Avicenna) has stated that, chronic liver ailment produces jaundice, dropsy and swelling at the belly and that the healthy status of the liver can be restored through a temporary diet of camel milk and male Arabian (Najib) camel urine, (AI-Nusaymi, 1988). Thus, Arabian camel urine was a standard prescription in Arabic medicine and remains to this day for chronic medical problems either by ingestion or topical application, it is useful in correcting digestive disorders in general, helping detoxify the liver in

particular and for treatment of hepatitis (Ibn Alirazag, 1206; Natali, 2002; Ohaj, 1993; 1998).

In-vivo designed series of experiments in which mixture of camel urine and milk was given to Albino mice showed no effects on the normal liver (Khalifa 1999), kidney (El-Elyani 1999), and stomach (El-Elyani and Khalifa 2006) which indicated the biological effect of the mixture as safe product.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials and experimental designs

Two experiments on rats were performed at Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum, in August 2011. The first experiment was run to determine the hepatoprotective effect of camel urine and the second experiment was done to study the hepatoprotective effect of the mixture of camel milk and urine.

2.1.1 Hepatoprotective effect of one humped camel urine (*Camelus dromedaries*) in rats:

2.1.1.1 Animals, housing and management

Twenty five Wister albino rats of both sexes weighting (85-105) gm were obtained from the Animal House, Faculty of Pharmacy, University of Khartoum. The rats were kept within the premises of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum. The rats were allowed one week adaptation period. They were housed in cages and maintained in a room under normal environmental conditions and controlled temperature ($28 \pm 2^{\circ}\text{C}$), relative humidity (60%) and light with free access to water and food (2.5 MCal and 20% crude protein rat formula feed). Rats were apparently healthy and they were identified by color tail marks.

2.1.1.2 Collection and preparation of camel urine

Fresh urine from young female camel (Bekra) (6 month up to 2 years old) was collected at early morning daily from Camel Research Centre Farm, Faculty of Veterinary Medicine, University of Khartoum.

The camel urine samples were collected during normal urination or by Tashweel technique which was done by touching the abdominal side of the female camel near the hide of the back leg (O'haj, 1998); by this technique urine samples could be available at any time.

2.1.1.3 Administration of camel urine to rats

At the end of the adaptation period, rats were divided randomly into 5 groups of 5 rats each. Group 1, the control, received only normal saline for 28 days (the experimental period). Group 2, (Ethanol control) received 10% Ethanol orally at 5g/kg body weight as single dose/day for 28 days. Group 3, received standard drug Silymarin, orally at 50 mg/kg body weight for the same period and after 3 hours 10% Ethanol was administered orally at 5g/kg. Group 4, received camel urine orally by dose of (1ml/100gm rats' body weight) for all period of the experiment (28 days). Group 5 received camel urine orally by dose of (1ml/100gm rats' body weight) and after 3 hours 10% Ethanol was administered orally at 5g/kg.

2.1.1.4 Blood samples and parameters

Blood samples were obtained from the orbital plexus of rats on day zero, day 15 and on day 29 of the experiment, for serum analysis and haematological examinations. The blood samples were collected successively into dry clean bottles and sera were harvested after centrifugation of blood for analyzing activities of the alkaline

phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), total protein, albumin and bilirubin concentrations.

Blood parameters include, haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs) counts, beside red blood cell indices which include, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Clinical signs were recorded; the specimens of 25 livers were collected immediately after slaughter of animals and were fixed in 10% buffered formal saline for histopathology examinations.

2.1.1.5 Hepatoprotective effect of one humped camel milk and urine (*Camelus dromedaries*) in rats:

2.1.1.5.1 Animals, housing and management

Twenty five Wister albino rats of either sex weighting (85-105) gm were obtained from the Animal House of the Veterinary Research Institute, Animal Resources Research Corporation, Soba, Khartoum. The rats were kept within the premises of the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Khartoum. The rats were allowed one week adaptation period. They were housed in cages and maintained in a room under standard environmental conditions and controlled temperature ($28 \pm 2^{\circ}\text{C}$), relative humidity (60%) and light with free access to water and food (2.5 MCal and 20% crude protein rat formula feed). Rats were apparently healthy and they were identified by color tail marks.

2.1.1.5.2 Collection and preparation of camel milk and urine

Early morning fresh milk from different females was brought from Camel Research Centre Farm, Faculty of Veterinary Medicine, University of Khartoum, and mixed with camel urine which collected as mentioned in the previous experiment.

2.1.1.5.3 Administration of camel milk and urine in rats

At the end of the adaptation period, rats were divided randomly into 5 groups of 5 rats each. Group 1, the control, received only normal saline for 28 days (the experimental period). Group 2, (Ethanol control) received 10% Ethanol orally at 5g/kg body weight as single dose/day for 28 days. Group 3, received standard drug Silymarin, orally at 50 mg/kg body weight for the same period and after 3 hours 10% Ethanol was administered orally at 5g/kg. Group 4, received camel milk mixed with camel urine (50:50) orally by dose of (2ml/100gm rats' body weight) for all period of the experiment (28 days). Group 5, received the same mixture of camel milk and urine orally by dose of (2ml/100gm rats' body weight) and after 3 hours 10% Ethanol was administered orally at 5g/kg.

2.1.1.5.4 Blood samples and parameters

Blood samples were obtained from the orbital plexus of rats on day zero, day 15 and on day 29 of the experiment, serum analysis and haematological examinations. The blood samples were collected successively into dry clean bottles and sera were harvested after centrifugation of blood for analyzing activities of the alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), total protein, albumin and billirubin concentrations.

Blood parameters include, haemoglobin (Hb) concentration, packed cell volume (PCV), red blood cells (RBCs) counts, beside red

blood cell indices which include, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC).

Clinical signs were recorded; the specimens of 25 livers were collected immediately after slaughter of animals and were fixed in 10% buffered formal saline for histopathology examinations.

2.2 Methods

2.2.1 Chemical methods used for determination of serum constituents

The sera were separated by centrifugation at 3000 r.p.m. for 5 minutes in a Hettich EBA 35 centrifuge and stored at -20°C Until analyzed. Spectrophotometer (Merck Mega, Version 0.6 1995 E Merck, Darmstadt, Germany) was used to record serum activities of enzymes AST, ALT, ALP and serum metabolites total protein, albumin, and billirubin.

2.2.1.1 Aspartate amino transferase (AST)

Serum AST activity was measured by commercial kit (Randox Laboratories Ltd, U.K). It is an enzymatic method that measures serum glutamyl oxaloacetic transaminase (SGOT) in serum according to (Retiman and Frankel, 1957).

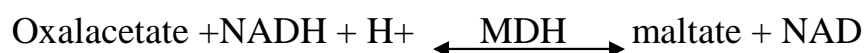
Principle

Aspartate aminotransferase catalyses the reversible transfer of an amino group aspartate to a-ketoglutarate and oxalacetate.

Reaction



The oxalacetate produced is reduced to maltate by maltate dehydrogenase (MDH) and NFDH.



The rate of decrease in concentration of NADH is proportional to the catalytic concentration of AST present in the serum sample.

Protocol

None-haemolysed serum was added to buffered substrate mixture of L-aspartate and α-oxoglutarate. The absorbance at a wave length of 365 nm was read at one minute intervals after mixing the serum with the buffered substrate solution. The AST was measured in IU/L. The mean absorbance change per minute (A₃₆₅/ minute) was used for calculation of enzyme activity as follows: IU=A₃₆₅ nm/minute × 2059.

2.2.1.2 Alanine amino transferase (ALT)

It is an enzymatic methods which measures serum glutamic pyruvic transaminase (SGPT) in serum according to (Retiman and Frankel, 1957).

Principle

Alanine amino transferase is measured by monitoring the concentration of pyruvic hydrazone formed with 2-4 dinitrophenylhydrazine.

Reaction



Protocol

None-haemolysed serum was added to buffered substrate mixture of L-aspartate and α-oxoglutarate. The absorbance of samples was read against the reagent blank after 5 minutes at wave length of 630 nm UV/VIS Spectrophotometer. The ALT was measured in IU/L. as follows: IU=A_{630nm}/minute ×2059.

2.2.1.3 Alkaline phosphatase (ALP)

It is an optimized method according to the recommendation of Chemie (1972).

Principle

In alkaline medium serum alkaline phosphatase (ALP), splits p-nitro-phenyl phosphatase in the presence of Mg^{+2} ions, into p-nitro-phenyl and phosphate. At the pH of the reaction, p-nitro-phenyl was coloured yellow; the optical density measured in a Spectrophotometer (Jenway 6305 UV/VIS) at the wave length 405 nm.

Reaction

P-nitrophenylphosphate + $H_2O \xrightarrow{ALT}$ inorganic phosphate + p-nitro phenol

Calculation

ALP is calculated as follows: $U/I = 2760 \times A_{405 \text{ nm/min}}$.

(A= the mean of sample absorbance reading).

2.2.1.4 Total protein

Total serum protein was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Principle

Colorimetric determination of total protein in serum is based on the Biuret reaction. The serum protein reacts with copper sulphate in the presence of sodium hydroxide. The Rochelle salt (K-Na-tartrate) contained in the Biuret reagent is utilized to keep the formed cupric hydroxide in solution which gives the blue color. The intensity of the color produced is proportional to the amount of protein in the sample.

The absorbance of the sample (A sample) and of the standard (A standard) were read against the reagent blank in the Spectrophotometer at a wave length of 545 nm. The total serum protein concentration (C) was calculated as follows:

$$C \text{ (mg/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times \text{concentration of the standard}$$

2.2.1.5 Albumin

Serum albumin was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Principle

The measurement of serum albumin is based on its quantitative binding to the indicator 5, 5-di-purple, BCP.

Serum was mixed with a buffered BCP reagent and the mixture was incubated for 2 minutes at room temperature. The absorbance of the sample (A sample) and of the standard (A standard) was measured against blank at a wave length of 600 nm albumin concentration (C) was calculated as follows:

$$C \text{ (g/dl)} = \frac{A \text{ sample}}{A \text{ standard}} \times \text{concentration of the standard}$$

2.2.1.6 Billirubin

Serum total billirubin was measured by a colorimetric method using a commercial kit (Randox Laboratories Ltd., U.K.).

Principle

Albumin bound billirubin is released by a detergent and the total billirubin reacts with 2,4 - di-chloroalanine to form a coloured

azobillirubin which is measured in the Spectrophotometer at a wave length of 456 nm.

Serum was mixed with the working reagent solution and the mixture was allowed to stand for 10 minutes protected from light. Absorbance of the sample (A-sample) was measured against the sample blank and concentration (C) of bilirubin was calculated as follows:

$$C \text{ (mg/dl)} = A \text{ sample} \times 12.5$$

2.2.2 Haematological methods

These were described by Schalm, (1965). Blood samples from ocular vein of rats were collected into dry clean bottles with anticoagulant ethylene diamine tetra acetic acid (EDTA).

2.2.2.1 Packed cell volume (PCV)

Blood samples were drawn into microhaematocrit capillary tubes and sealed at one end with cristaseal (Hawksley). The capillary tubes were centrifuged for 5 minutes in microhaematocrit centrifuged (Hawksley and Sons, Ltd. England) and the (PCV) percentage was determined from the scale on the Hawksley microhaematocit reader.

2.2.2.2 Haemoglobin (Hb) concentration

The concentration of haemoglobin (Hb) was measured by the cyanmethaemoglobin technique (Kelly, 1984) using Colorimeter (Ciba Corning, model 252).

A volume of 0.02 ml of blood was added to the 4 ml of Drabkins solutions (0.2 gm potassium cyanide, 0.2 potassium ferri cyanide and 1gm bicarbonate per liter of distilled water).

After 10 minutes, the haemoglobin (Hb) concentration measured in g/dl of blood. The method is based on the conversion of haemoglobin by Drabkin solution to cyanmethaeglobin.

2.2.2.3 Total Red blood cells (RBCs) count

Total erythrocytes were counted by using Neubauer haemocytometer (Hawksly and Sons Ltd, England) and Hayem's solution (Kelly, 1984) as a diluents consisting of (0.5 gm sodium sulphate, 0.5 mercuric chloride and 1 gm sodium chloride) made up to 200 ml with distilled water.

Calculation

$$200 \times 50 \times R \text{ cells} = 10.000 \times R. \text{ ul.}$$

2.2.2.4 Mean corpuscular volume (MCV)

The mean volume of the red corpuscle was calculated from the erythrocyte count and the volume of packed red cells as described by Maxwell and Wintrobe, (1967) as follows:

$$\text{MCV} = \frac{\text{Volume of packed red cells, ml per 1000ml}}{\text{Red cells count, millions per cm}} \times 100$$

2.2.2.5 Mean corpuscular haemoglobin (MCH)

Mean corpuscular haemoglobin (MCH) is a calculation of the average amount of oxygen-carrying hemoglobin inside a red blood cell. It is calculated by dividing the total mass of hemoglobin by the number of red blood cells in a volume of blood.

$$\text{MCH} = \frac{\text{haemoglobin /gm per 100 ml}}{\text{Red cells count, millions per cm}} \times 100$$

2.2.2.6 Mean corpuscular haemoglobin concentration (MCHC)

MCHC was calculated when the Hb concentration and the volume of packed red cells are known (Maxweil and Wintrobe, 1967).

Calculation

$$\text{MCHC} = \frac{\text{haemoglobin /gm per 100 ml}}{\text{Volume of packed red cells, ml per 100 ml}} \times 100$$

2.2.3 Histopathological methods

The postmortem examination was carried out immediately after slaughtering of the animals. Organs were examined for the presence of abscesses and lesions. The specimens of the liver were collected and fixed in 10% buffered formal saline, and transported to the laboratory for histopathological processing and investigation. Specimens embedded in paraffin wax sectioned at 5µm and stained routinely with Haemotoxylin and Eosin (H and E) using Mayere haemalum.

2.2.4 Statistical analysis

Data were entered and analyzed using SAS statistical package. Numerical data were expressed as means and standard errors. Significance of difference between means was tested by one-way ANOVA, depending on the number of compared groups; with a p value of = 0.05 considered statistically significant. Changes in individual serum parameters against time were figured out as histograms and compared (Mendelhall, 1971).

CHAPTER THREE

RESULTS

3.1. Effect of camel urine against ethanol induced liver damage in rats

3.1.1 Clinical signs

Rats in group 2 which treated with ethanol showed depression and nervous signs when compared to the control rats, while there were no clinical signs observed in rats of group 3 (intoxicated with ethanol and treated with Silymarin), group 4 (treated with urine alone) or group 5 (treated with ethanol and camel urine).

3.1.2 Post-mortem findings

The livers of rats of group 2 (ethanol group), showed fatty changes, congestion and adhesion of lobes, while there were no pathological changes observed in the livers of group 3 (Silymarin + ethanol) or group 4 (the urine group). In group 5 (Ethanol + Urine) the liver showed slight fatty change.

3.1.3 Changes in serum enzymes

The effect of camel urine on the activities of enzymes AST, ALT, and ALP in the serum of rats are clear in Table (1), and Figures (1)-(3).

In rats treated with ethanol (group 2), the activities of enzymes AST, ALT, and ALP were significantly increased when compared with the control group (group 1).

Administration of camel urine to rats in group 5 which also treated with ethanol resulted in significant fall in the levels of the enzymes AST, ALT, and ALP, when compared to the group treated with ethanol.

Table (1): Changes in serum enzymes constituents of rats treated with camel urine and ethanol

Groups	AST (SGOT) U/I (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	38.68 \pm 1.32 a	37.26 \pm 1.15 c	37.8 \pm 0.73 d
Group2	37.36 \pm 1.65 a	63.8 \pm 3.02 a	62.6 \pm 2.01 a
Group3	35.34 \pm 0.83 a	53.2 \pm 1.98 b	54.2 \pm 2.27 b
Group4	36.68 \pm 1.06 a	49.46 \pm 1.89 b	47.2 \pm 2.13 c
Group5	37.3 \pm 1.63 a	52.6 \pm 1.63 b	52.6 \pm 2.25 bc
Groups	ALT (SGPT) U/I (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	5.2 \pm 1.02 a	6 \pm 1 c	6.6 \pm 0.51 d
Group2	3.8 \pm 0.49 a	15.4 \pm 0.87 a	16.8 \pm 0.86 a
Group3	3.4 \pm 0.68 a	14.8 \pm 0.37 a	16.2 \pm 0.37 ab
Group4	3.4 \pm 0.93 a	10.2 \pm 1.07 b	9.4 \pm 0.75 c
Group5	5.8 \pm 1.32a	13.4 \pm 0.51 a	14.8 \pm 0.58 b
Groups	ALP U/I (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	80.4 \pm 1.03 a	76 \pm 1 b	75.8 \pm 1.07 b
Group2	79.2 \pm 1.39 a	79.4 \pm 0.51 a	79.8 \pm 0.66 a
Group3	80 \pm 1 a	78 \pm 0.71 ab	77.8 \pm 0.37 ab
Group4	77.8 \pm 0.86 a	79.2 \pm 1.66a	77.6 \pm 1.29ab
Group5	80 \pm 0.55 a	75.8 \pm 0.86 b	77 \pm 0.45 b

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

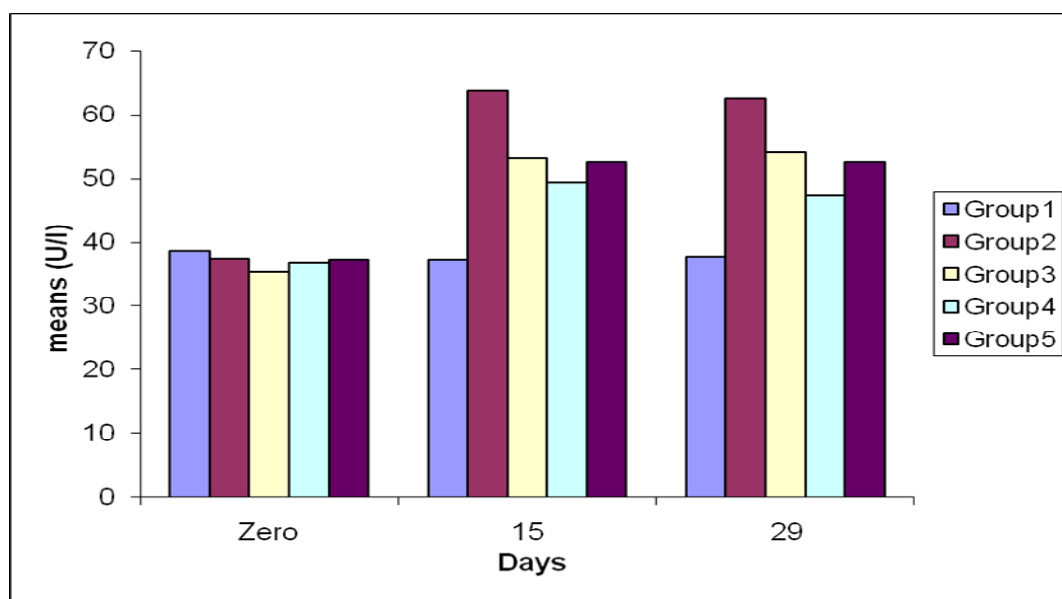


Fig. (1) Changes in serum (AST) levels of rats treated with camel urine and ethanol

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

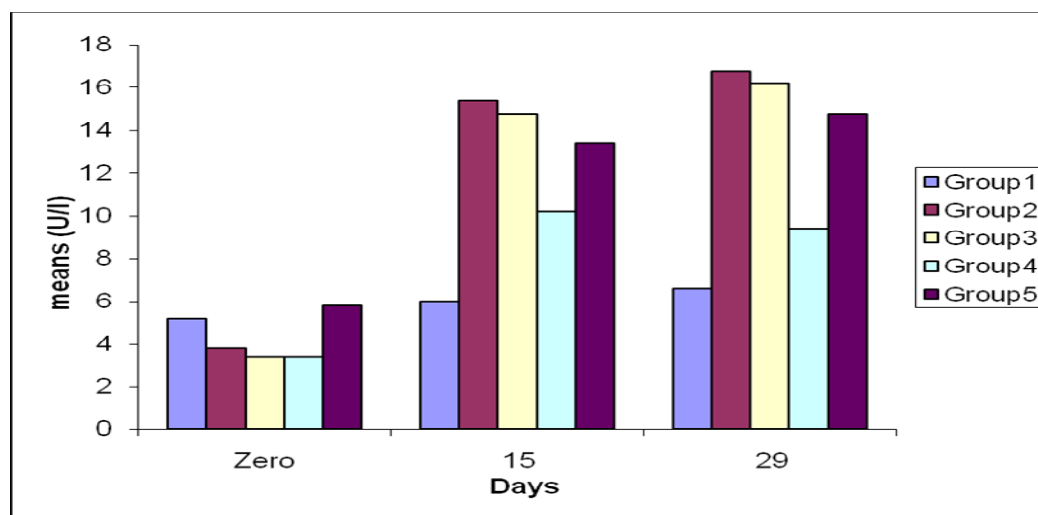


Fig. (2) Changes in serum (ALT) levels of rats treated with camel urine and ethanol

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

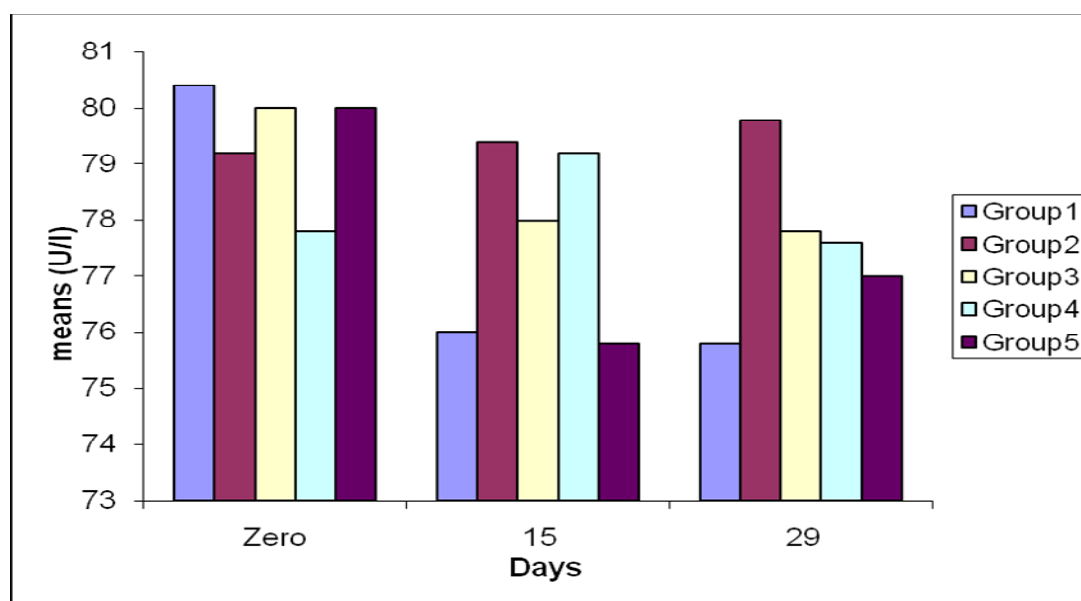


Fig. (3) Changes in serum ALP levels of rats treated with camel urine and ethanol

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

3.1.4 Changes in serum metabolites parameters

Table (2) and Figures (4)-(6), summarized the effect of camel urine on the concentration of the metabolites, total protein, albumin and billirubin in the serum of rats.

In rats treated with ethanol (group 2), the concentration of the metabolites total protein, albumin and billirubin were significantly increased when compared with the control group (group 1).

Administration of camel urine to rats in group 5 which also treated with ethanol resulted in significant fall in the concentration of the metabolites, total protein, albumin and billirubin, when compared to the group treated with ethanol.

3.1.5 Changes in haematological parameters

The mean values of haematological parameters, PCV, Hb and red blood cells count, are presented in table (3). The mean values of PCV showed no significant difference in all groups at day Zero and day (15), but at day (29) there was significant increase in the mean values of PCV in group4 (urine group) and group5 (urine + ethanol), when compared to the ethanol group (group 2).

The mean values of Hb showed no significant change in all groups at day Zero of the experiment, but the mean values of Hb at day (15) and day (29) showed significant increase in group 4 (urine group), group 5 (ethanol + urine) and group 3 (Silymarin group), which showed a little decrease at day (15), when compared to the ethanol group.

The mean values of RBC at day (15) showed significant increase in group 4 (urine group), beside a little decrease in group 5 (ethanol + urine) and group 3 (Silymarin group), when compared to the ethanol group, while the mean values of RBC showed no significant change in all groups at day (29) of the experiment.

Table (2): Changes in serum metabolites concentration of rats treated with camel urine and ethanol

Groups	Total Protein g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	5.38 \pm 0.21 a	5.42 \pm 0.12 a	5.32 \pm 0.22 a
Group2	5.54 \pm 0.12 a	5.66 \pm 0.09 a	5.7 \pm 0.07 a
Group3	5.58 \pm 0.09 a	5.66 \pm 0.09 a	5.6 \pm 0.05 a
Group4	5.7 \pm 0.07 a	5.68 \pm 0.11 a	5.6 \pm 0.15 a
Group5	5.52 \pm 0.09 a	5.52 \pm 0.09 a	5.48 \pm 0.04 a
Groups	Albumin g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	2.78 \pm 0.097 a	2.42 \pm 0.12 b	2.46 \pm 0.14 b
Group2	2.62 \pm 0.07 a	2.74 \pm 0.07 a	2.76 \pm 0.05 a
Group3	2.6 \pm 0.07 a	2.72 \pm 0.07 a	2.7 \pm 0.05 ab
Group4	2.7 \pm 0.045 a	2.76 \pm 0.09 a	2.56 \pm 0.09ab
Group5	2.62 \pm 0.09 a	2.58 \pm 0.07 ab	2.60 \pm 0.03 ab
Groups	Billirubin mg/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	0.52 \pm 0.14 a	0.3 \pm 0.07 c	0.22 \pm 0.04 c
Group2	0.42 \pm 0.14 a	0.54 \pm 0.05ab	0.56 \pm 0.05 a
Group3	0.28 \pm 0.13 a	0.38 \pm 0.04 bc	0.46 \pm 0.05 ab
Group4	0.16 \pm 0.02 a	0.6 \pm 0.07 a	0.58 \pm 0.04 a
Group5	0.48 \pm 0.18 a	0.40 \pm 0.04 bc	0.38 \pm 0.04 b

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

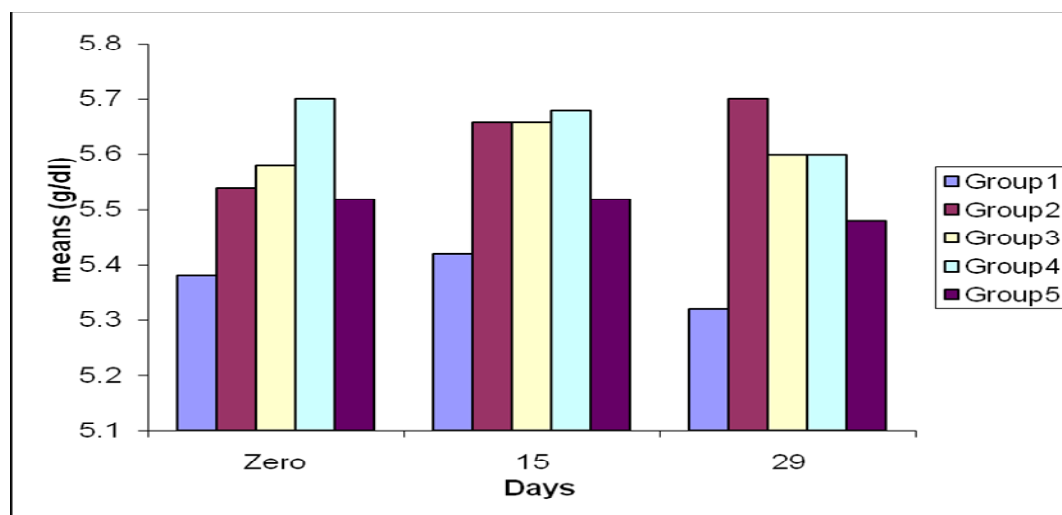


Fig. (4) Changes in total protein concentration in rats treated with camel urine and ethanol

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

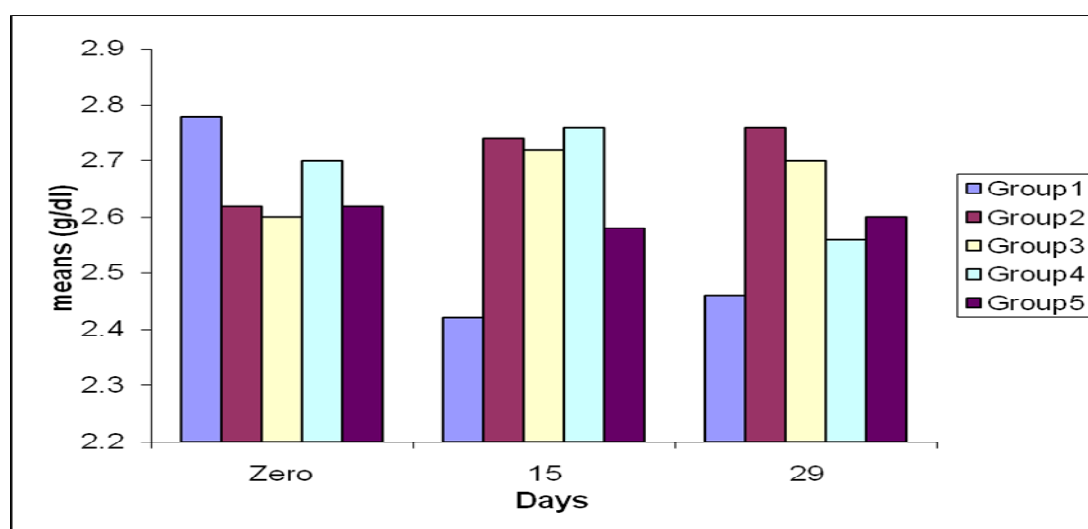


Fig. (5) Changes in albumin concentration in rats treated with camel urine and ethanol

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

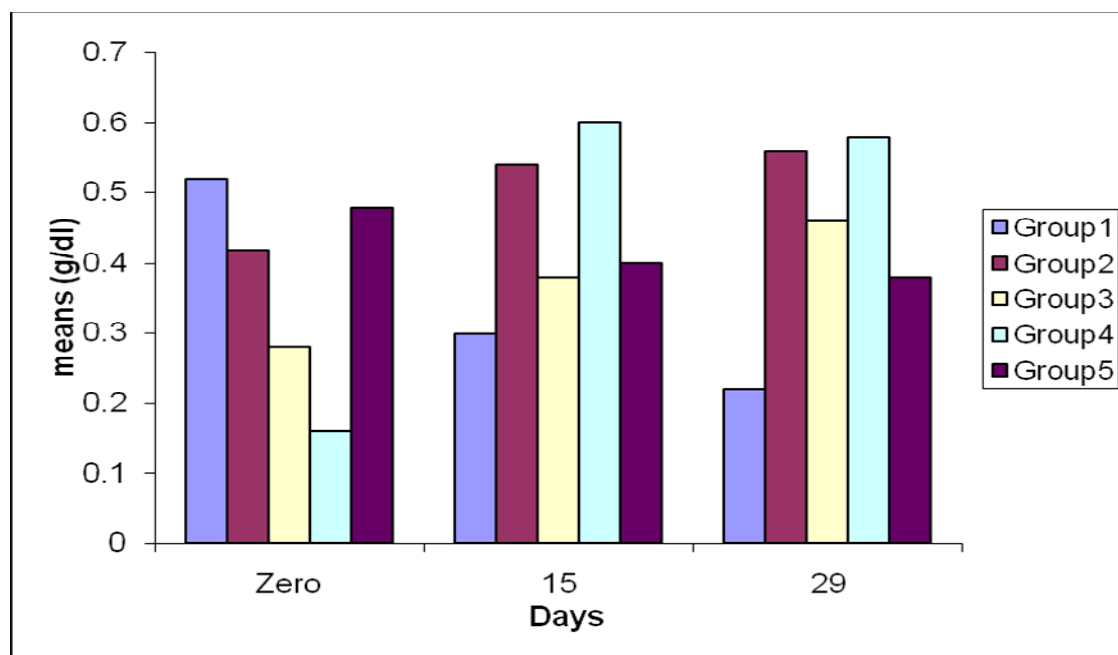


Fig. (6) Changes in bilirubin concentration in rats treated with camel urine and ethanol

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

Table (3): Changes in haematological parameters in rats treated with camel urine and ethanol

Groups	PCV % (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	32.4 \pm 1.6 a	36.8 \pm 0.86 a	36.8 \pm 1.53 b
Group2	34 \pm 0.45 a	35.2 \pm 1.43 a	36.6 \pm 1.44 b
Group3	32.2 \pm 2.48 a	34.4 \pm 0.75 a	38.4 \pm 1.63 b
Group4	34.2 \pm 1.16 a	33.8 \pm 1.24 a	41.6 \pm 1.44 a
Group5	32.8 \pm 0.86 a	33.8 \pm 0.73 a	40.2 \pm 0.66 a
Groups	Hb g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	16.28 \pm 0.42 b	16.1 \pm 0.47 a	16 \pm 0.85 a
Group2	16 \pm 0.44 b	12.4 \pm 0.76 c	12.3 \pm 1.83 b
Group3	17 \pm 1.03 b	11.7 \pm 0.60 c	12.98 \pm 0.83ab
Group4	16.92 \pm 0.50 b	15.06 \pm 0.75ab	15.3 \pm 0.72 ab
Group5	18.4 \pm 0.91 b	13.2 \pm 0.64 bc	14.1 \pm 0.4 ab
Groups	RBC $\times 10^6$ (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	4.96 \pm 0.20 ab	5.96 \pm 0.18 a	4.15 \pm 0.85 b
Group2	4.74 \pm 0.24 b	4.66 \pm 0.19 c	4.24 \pm 0.12 b
Group3	4.7 \pm 0.24 b	4.12 \pm 0.12 d	4.42 \pm 0.19 b
Group4	5.18 \pm 0.24 ab	5.12 \pm 0.12 b	4.86 \pm 0.31 b
Group5	5.5 \pm 0.15 a	4.58 \pm 0.07 c	4.72 \pm 0.19 b

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

3.1.6 Changes in red blood cell indices values

The changes in red blood indices MCV, MCH and MCHC, are presented in Table (4). The mean values of MCV showed no significant difference in all groups at day Zero and day (29) of the experiment, but at day (15) there was significant increase in group 3 (Silymarin group) and significant decrease in group 4 (urine group), when compared to the ethanol group.

The mean values of MCH and MCHC showed no significant difference in all groups throughout the experimental period. But the mean values of MCHC at day (15) and day (29) showed significant increase in group 4 (urine group) and group 5 (ethanol + urine), beside significant decrease in group 3 (Silymarin group) at day (15) and increase at day (29), when compared to the ethanol group.

3.1.7 Histopathological changes

Histopathology of the liver sections treated with camel urine were presented in Figure (7) (a-d). In group 2 (ethanol group) liver damage was seen in a form of generalized necrosis, fatty change and congestion, in the group treated with urine alone, there was generalized fatty changes and slight necrosis, but in the group treated with ethanol and urine there was generalized fatty change, while in the group treated with ethanol and Silymarin there was slight fatty change, while there were no pathological changes seen in the livers of control group.

Table (4): Changes in values of red blood cell indices in rats treated with camel urine and ethanol

Groups	MCV fl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	65.24 \pm 4.14 a	59.28 \pm 1.74 d	81.44 \pm 2.58 a
Group2	73.56 \pm 3.76 a	75.64 \pm 1.82 b	86.46 \pm 3.59 a
Group3	66.34 \pm 4.68 a	83.62 \pm 1.65 a	87.56 \pm 4.14 a
Group4	68.02 \pm 3.98 a	64.72 \pm 1.13 c	86.46 \pm 3.96 a
Group5	61.26 \pm 1.93 a	74.02 \pm 1.48 b	85.86 \pm 4.57 a
Groups	MCH pg (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	33.64 \pm 1.45 a	27.82 \pm 0.73 a	35.44 \pm 1.53 a
Group2	34.9 \pm 2.24 a	26.56 \pm 0.95 a	29.24 \pm 4.50 a
Group3	34.64 \pm 2.29 a	28.34 \pm 0.72a	29.48 \pm 1.90 a
Group4	32.86 \pm 1.44 a	28.92 \pm 0.75 a	31.60 \pm 1.16 a
Group5	33.64 \pm 2.25 a	28.88 \pm 1.60 a	30.02 \pm 1.06 a
Groups	MCHC g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	50.88 \pm 1.69 a	43.76 \pm 1.45 a	41.48 \pm 2.03 a
Group2	49.48 \pm 2.58 a	35.08 \pm 1.22 b	33.4 \pm 4.52 b
Group3	49.38 \pm 2.91 a	33.94 \pm 1.25 b	33.52 \pm 0.75 b
Group4	50.20 \pm 1.65 a	37.92 \pm 3.85ab	37.06 \pm 1.55ab
Group5	54.92 \pm 3.81 a	37.2 \pm 3.64 ab	35.1 \pm 1.05ab

Group1 (Control), Group2 (10% ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), Group4 (camel urine at "1ml/100gm rats' B.W."), Group5 (camel urine at "1ml/100gm rats' B.W." + 10% ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

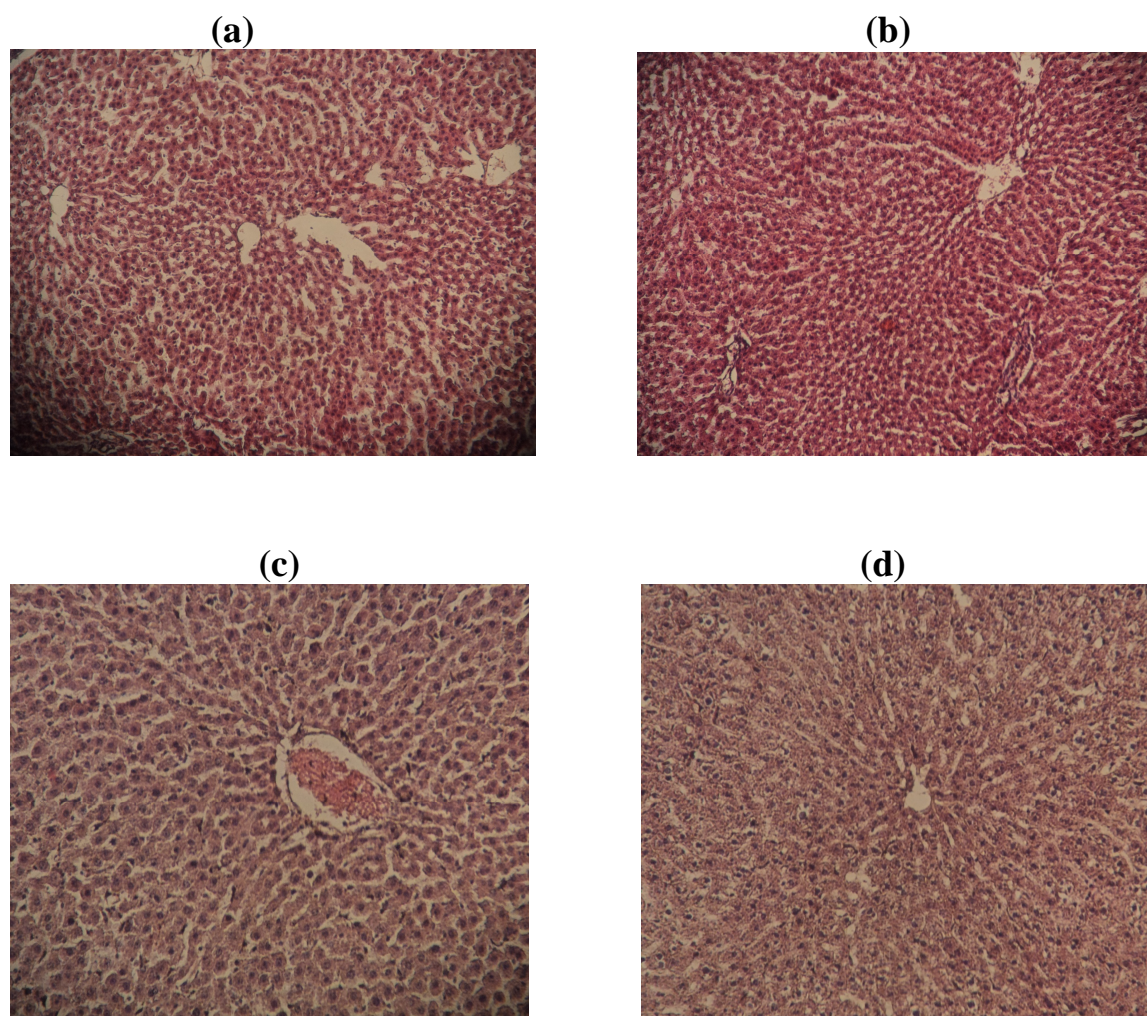


Fig. (7) Histopathological changes in livers of rats treated with ethanol, camel urine and Silymarin

- (a) = Section of liver cells of group 2 (10% ethanol at 5g/kg), showing liver damage in a form of generalized necrosis, fatty change and congestion.
- (b) = Section of liver cells of group 4 treated with camel urine at "1ml/100gm B. W.", showing generalized fatty changes and slight necrosis.
- (c) = Section of liver cells of group 5 which treated with camel urine at "1ml/100gm B. W." + 10% ethanol at 5g/kg, showing generalized fatty change.
- (d) = Section of liver cells of group 3 which treated with Silymarin at 50 mg/kg + 10% ethanol at 5g/kg), showing slight fatty change.

3.2 Effect of camel milk and urine against ethanol induced liver damage in rats

3.2.1 Clinical Signs

There were no obvious clinical signs observed in the groups treated with Silymarin, milk and urine or milk and urine with ethanol (group 3, 4 and 5, respectively). But there were depression and general unthriftiness in the group treated with ethanol (group 2) which occurred after 13 days from the beginning of experiment. No clinical signs observed in the control group.

3.2.2 Post-mortem findings

At necropsy, in group 2 (ethanol group), the livers of the rats showed fatty changes, slight congestion and adhesion in lobes, while in group 1 (control), group 4 (Milk and urine) and group 5 (milk and urine + ethanol) no post mortem changes were observed in internal organs. In group 3 (Silymarin) there was fatty change.

3.2.3 Changes in serum enzymes

Table (5) and Figures (8)-(10) summarized the effect of camel milk and urine on the activities of serum enzymes AST, ALT, and ALP.

In rats intoxicated with ethanol (group 2), the activities of enzymes AST, ALT, and ALP were significantly increased when compared with the untreated groups 1 (the control).

Administration of camel milk mixed with camel urine to rats in group 5 which was intoxicated with ethanol resulted in significant amelioration of the levels of the enzymes AST, ALT, and ALP, when compared to the group treated with ethanol.

Table (5): Changes in serum enzymes constitutes of rats treated with mixture of camel milk and urine plus ethanol

Groups	AST (SGOT) U/I (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	39.34 \pm 1.23 a	37.26 \pm 1.15 c	38.4 \pm 0.98 c
Group2	38.02 \pm 1.71ab	63.8 \pm 3.02 a	62.6 \pm 2.01 a
Group3	35.34 \pm 0.83 b	53.2 \pm 1.98 b	54.2 \pm 2.27 b
Group4	36.02 \pm 0.68ab	38.08 \pm 1.37 c	37.4 \pm 0.87 c
Group5	35.34 \pm 0.83b	51.8 \pm 2.06 b	50.4 \pm 1.69 b
Groups	ALT (SGPT) U/I (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	5 \pm 1.14 a	6 \pm 0.71 c	6.4 \pm 0.51 c
Group2	4.6 \pm 0.75 a	15.4 \pm 0.87 a	16.8 \pm 0.86 a
Group3	3.4 \pm 0.68 a	14.8 \pm 0.37 a	16.2 \pm 0.37 a
Group4	4.6 \pm 0.74 a	5.4 \pm 0.93 c	5.6 \pm 0.51 c
Group5	2.6 \pm 0.24 a	12.6 \pm 0.68 b	14.2 \pm 0.37 b
Groups	(ALP) U/I (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	80.4 \pm 2.06 a	76.2 \pm 1.39 bc	76.2 \pm 1.24 b
Group2	79.2 \pm 1.16 a	79.4 \pm 0.51 a	79.8 \pm 0.66 a
Group3	79.8 \pm 1.16 a	78 \pm 0.70 ab	77.8 \pm 0.37 ab
Group4	78.4 \pm 1.44 a	77 \pm 0.71 abc	76.8 \pm 0.58 b
Group5	77.6 \pm 0.93 a	74.8 \pm 0.37 c	76.2 \pm 0.37 b

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

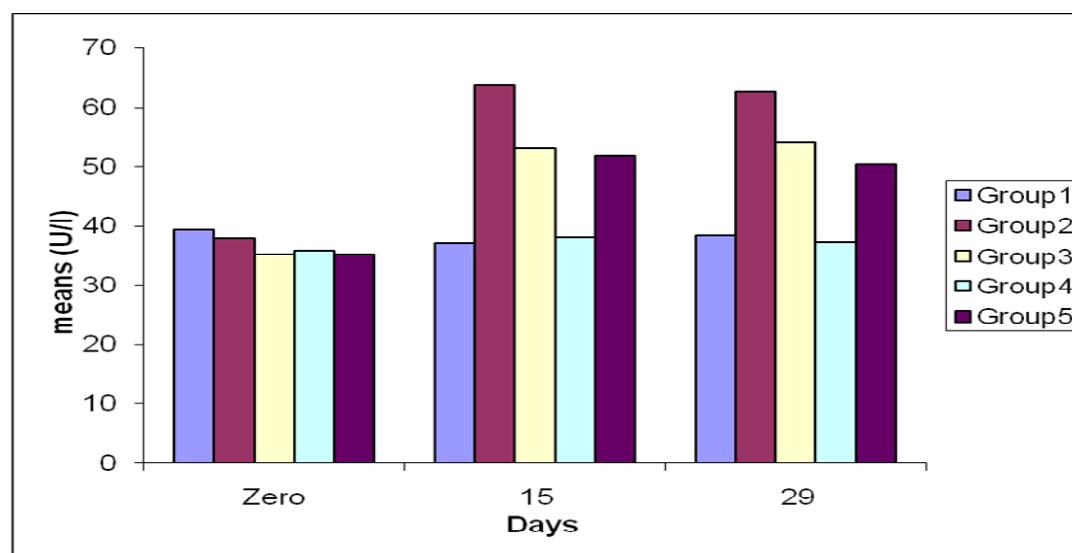


Fig. (8) Changes in Serum (AST) SGOT levels of rats treated with mixture of camel milk and urine plus ethanol

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

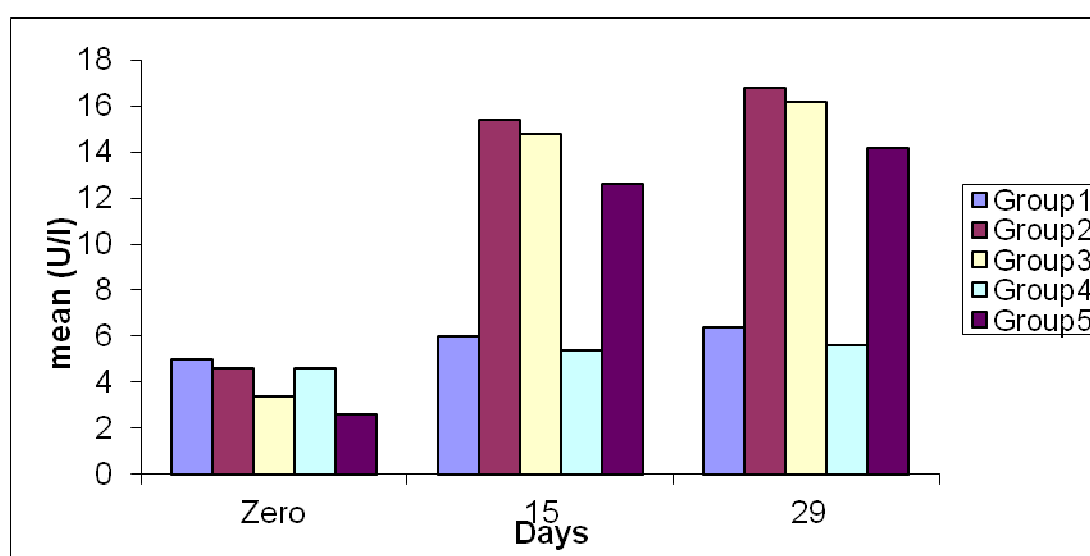


Fig. (9) Changes in Serum (ALT) SGPT levels of rats treated with mixture of camel milk and urine plus ethanol

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

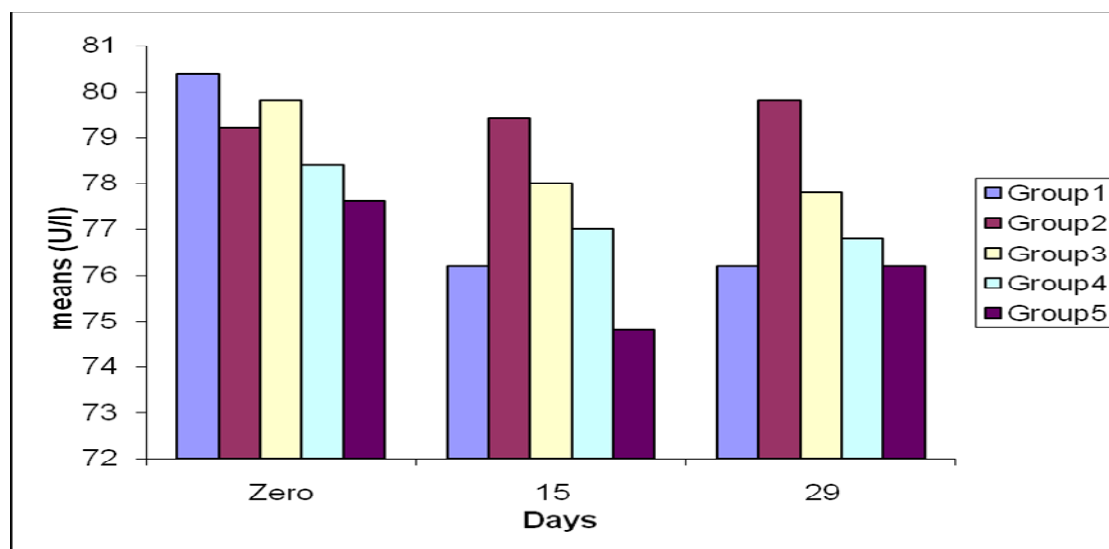


Fig. (10) Changes in Serum ALT levels of rats treated with mixture of camel milk and urine plus ethanol

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

3.2.4 Changes in serum metabolites parameters

The effect of the camel milk and urine on the concentration of the metabolites total protein, albumin and billirubin in the serum of rats are clear in Table (6) and Figures (11)-(13).

In rats treated with ethanol only (group 2), the concentration of the metabolites total protein, albumin and billirubin were significantly increased when compared with the untreated groups (the control).

Administration of camel milk mixed with camel urine to rats intoxicated with ethanol (group 5) resulted in significant decrease in the concentration of the metabolites total protein, albumin and billirubin, when compared to the group treated with ethanol.

3.2.5 Determination of haematological parameters

Table (7) presented the mean value of haematological parameters, PCV, Hb and RBC. The mean values of PCV fluctuate within the normal range during the period of the experiment in all groups and showed no significant difference between the test and control groups in all days of the experiment.

The mean values of Hb and RBC showed no significant difference between the treated groups and ethanol group at day Zero of the experiment, but there was significant increase in Hb and RBC values in group 4 (milk and urine), at day (15), and day (29) of the experiment.

3.2.6 Calculation of red blood cell indices

The changes in red blood indices MCV, MCH and MCHC, are presented in Table (8). The mean values of MCV, MCH and MCHC fluctuate within the normal range and showed no significant difference between the treated and control group, in all groups throughout the experimental period.

Table (6): Changes in serum metabolites concentrations of rats treated with mixture of camel milk and urine plus ethanol

Groups	Total Protein g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	5.5 \pm 0.17 a	5.26 \pm 0.14 b	5.26 \pm 0.15 c
Group2	5.46 \pm 0.12 a	5.66 \pm 0.09 a	5.7 \pm 0.07 a
Group3	5.58 \pm 0.09 a	5.66 \pm 0.09 a	5.6 \pm 0.05 ab
Group4	5.58 \pm 0.10 a	5.36 \pm 0.10 ab	5.36 \pm 0.05 bc
Group5	5.6 \pm 0.05 a	5.5 \pm 0.07 ab	5.42 \pm 0.06 bc
Groups	Albumin g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	2.78 \pm 0.10 a	2.34 \pm 0.15 b	2.48 \pm 0.14 bc
Group2	2.58 \pm 0.13 a	2.74 \pm 0.07 a	2.76 \pm 0.05 a
Group3	2.6 \pm 0.07 a	2.72 \pm 0.07 a	2.7 \pm 0.05 ab
Group4	2.68 \pm 0.10 a	2.5 \pm 0.12 ab	2.42 \pm 0.06 c
Group5	2.6 \pm 0.05 a	2.6 \pm 0.07 ab	2.5 \pm 0.05 bc
Groups	Billirubin mg/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	0.58 \pm 0.13 a	0.3 \pm 0.07 b	0.22 \pm 0.04 c
Group2	0.4 \pm 0.15 ab	0.54 \pm 0.05 a	0.56 \pm 0.05 a
Group3	0.28 \pm 0.13 ab	0.38 \pm 0.04 b	0.46 \pm 0.05 ab
Group4	0.14 \pm 0.02 b	0.3 \pm 0.04 b	0.28 \pm 0.04 c
Group5	0.32 \pm 0.15 ab	0.32 \pm 0.04 b	0.34 \pm 0.02 bc

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

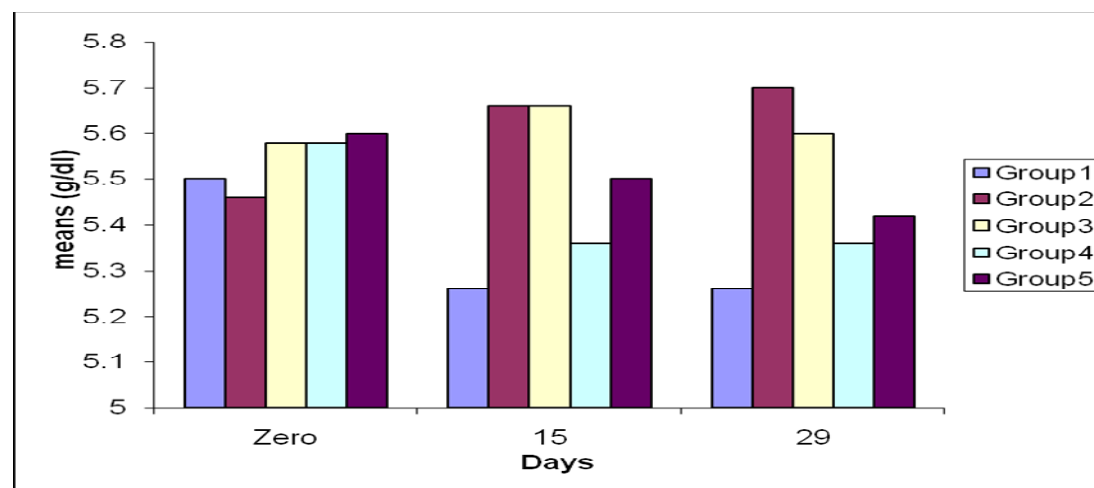


Fig. (11) Changes in total protein concentration in rats treated with mixture of camel milk and urine plus ethanol

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

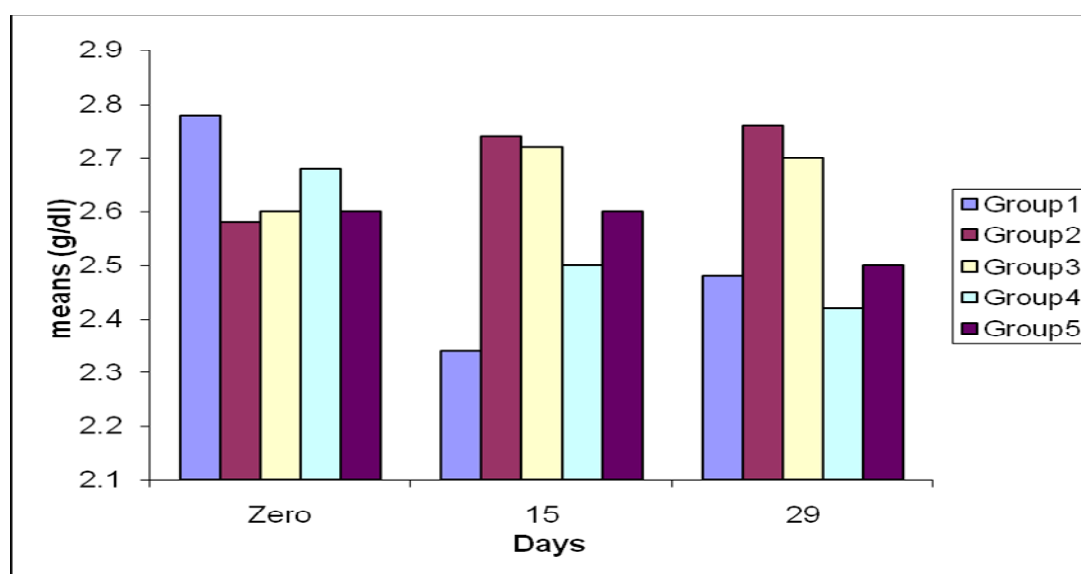


Fig. (12) Changes in albumin concentration in rats treated with mixture of camel milk and urine plus ethanol

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

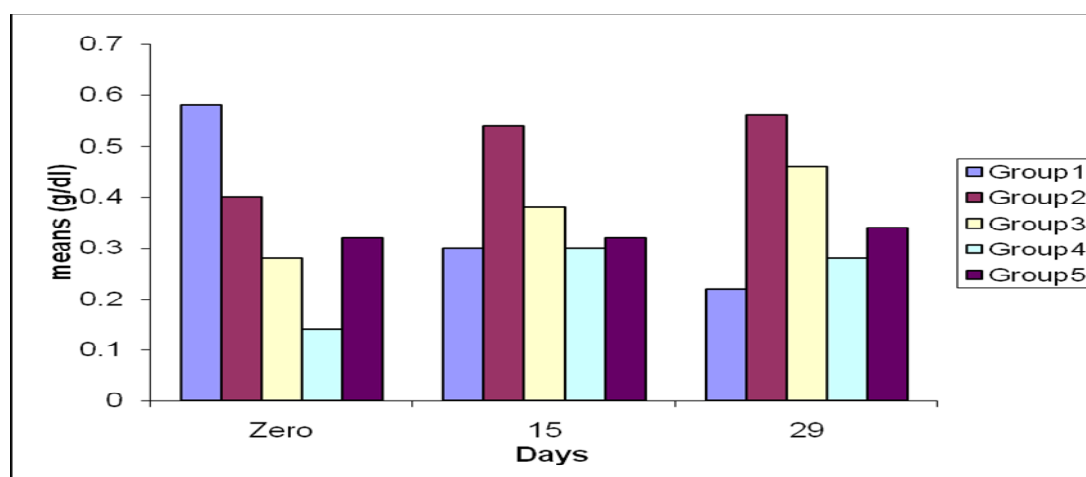


Fig. (13) Changes in bilirubin concentration of rats treated with mixture of camel milk and urine plus ethanol

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

Table (7): Changes in haematological parameters in rats treated with mixture of camel milk and urine plus ethanol

Groups	PCV % (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	32.6 \pm 1.36 a	37.8 \pm 1.16 a	36.4 \pm 2.93 a
Group2	33.6 \pm 1.50 a	35.2 \pm 1.43 ab	36.6 \pm 1.44 a
Group3	30.6 \pm 2.98 a	34.4 \pm 0.75 ab	38.4 \pm 1.63 a
Group4	34 \pm 3.41 a	32.6 \pm 2.50 b	38.2 \pm 1.07 a
Group5	37.4 \pm 2.01a	35.2 \pm 0.86 ab	38.6 \pm 2.23 a
Groups	Hb g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	16.5 \pm 0.57 a	15.96 \pm 0.51 a	16.7 \pm 1.25 a
Group2	16.92 \pm 0.50 a	12.4 \pm 0.76 b	12.3 \pm 1.83 b
Group3	17.4 \pm 1.12 a	11.7 \pm 0.60 b	12.98 \pm 0.83 ab
Group4	16.28 \pm 0.42 a	13.6 \pm 0.51 b	16.5 \pm 0.27 a
Group5	15.24 \pm 1.41a	12.4 \pm 0.58 b	13.3 \pm 1.12 ab
Groups	RBC $\times 10^6$ (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	4.9 \pm 0.29 a	5.86 \pm 0.19 a	4.52 \pm 0.19 b
Group2	4.62 \pm 0.17 a	4.66 \pm 0.19 bc	4.24 \pm 0.12 b
Group3	4.8 \pm 0.28 a	4.12 \pm 0.12 c	4.42 \pm 0.19 b
Group4	4.64 \pm 0.58 a	4.9 \pm 0.28 b	5.7 \pm 0.27 a
Group5	4.96 \pm 0.26 a	4.52 \pm 0.22 bc	4.28 \pm 0.20 b

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

Table (8): Changes in values of red blood cell indices in rats treated with mixture of camel milk and urine plus ethanol

Groups	MCV fl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	65.4 \pm 3.57 a	65.16 \pm 3.71 b	85.66 \pm 3.50 a
Group2	73.5 \pm 3.57 a	75.64 \pm 1.82 ab	86.46 \pm 3.59 a
Group3	67.28 \pm 4.82 a	83.62 \pm 1.65 a	87.56 \pm 4.14 a
Group4	72.9 \pm 3.90 a	67.04 \pm 5.17 b	68.46 \pm 5.15 b
Group5	75.28 \pm 7.90 a	78.44 \pm 3.5 a	90.22 \pm 2.14 a
Groups	MCH pg (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	33.36 \pm 2.18 a	26.72 \pm 1.42 a	37.72 \pm 3.74 a
Group2	36.92 \pm 2.59 a	26.56 \pm 0.95 a	29.24 \pm 4.50 a
Group3	36.94 \pm 3 a	28.34 \pm 0.73 a	29.48 \pm 1.90 a
Group4	31.66 \pm 2.82 a	28.56 \pm 1.56 a	29.92 \pm 1.05 a
Group5	30.8 \pm 4.26 a	27.5 \pm 0.87 a	30.84 \pm 1.54 a
Groups	MCHC g/dl (Means \pm S.E)		
	Day Zero	Day 15	Day 29
Group1	51.4 \pm 1.26 ab	43.1 \pm 2.13 a	47.8 \pm 6.01 a
Group2	52.38 \pm 2.09 ab	35.08 \pm 1.22 b	33.4 \pm 4.52 b
Group3	55.82 \pm 7.97 a	33.94 \pm 1.25 b	33.52 \pm 0.75 b
Group4	39.96 \pm 5.25 b	43.76 \pm 2.81 a	43.4 \pm 1.88 ab
Group5	40.96 \pm 3.73 ab	35.22 \pm 1.45 b	34.22 \pm 1.14 b

Group1 (Control), Group2 (10% Ethanol at 5g/kg), Group3 (Silymarin at 50 mg/kg + 10% Ethanol at 5g/kg), Group4 (50:50 mixture of camel milk and urine at "2ml/100gm B. W."), Group5 (50:50 mixture of camel milk and urine at "2ml/100gm B. W." + 10% Ethanol at 5g/kg).

Means in the same column with the same letters are not significantly different.

3.2.7 Histopathological changes

Histopathology of the liver sections treated with camel milk and urine was presented in Figure (14) (a-d). In group 1 (the control) no pathological changes were seen. In group 2 (ethanol group) liver damage was seen in a form of centrilobular necrosis and lymphocytic infiltration, in the group treated with mixture of camel milk and urine, there was almost normal architecture, and in the group treated with ethanol plus camel milk and urine there was slight fatty change and lymphocytic infiltration, while in the group treated with ethanol and Silymarin there was generalized fatty change.

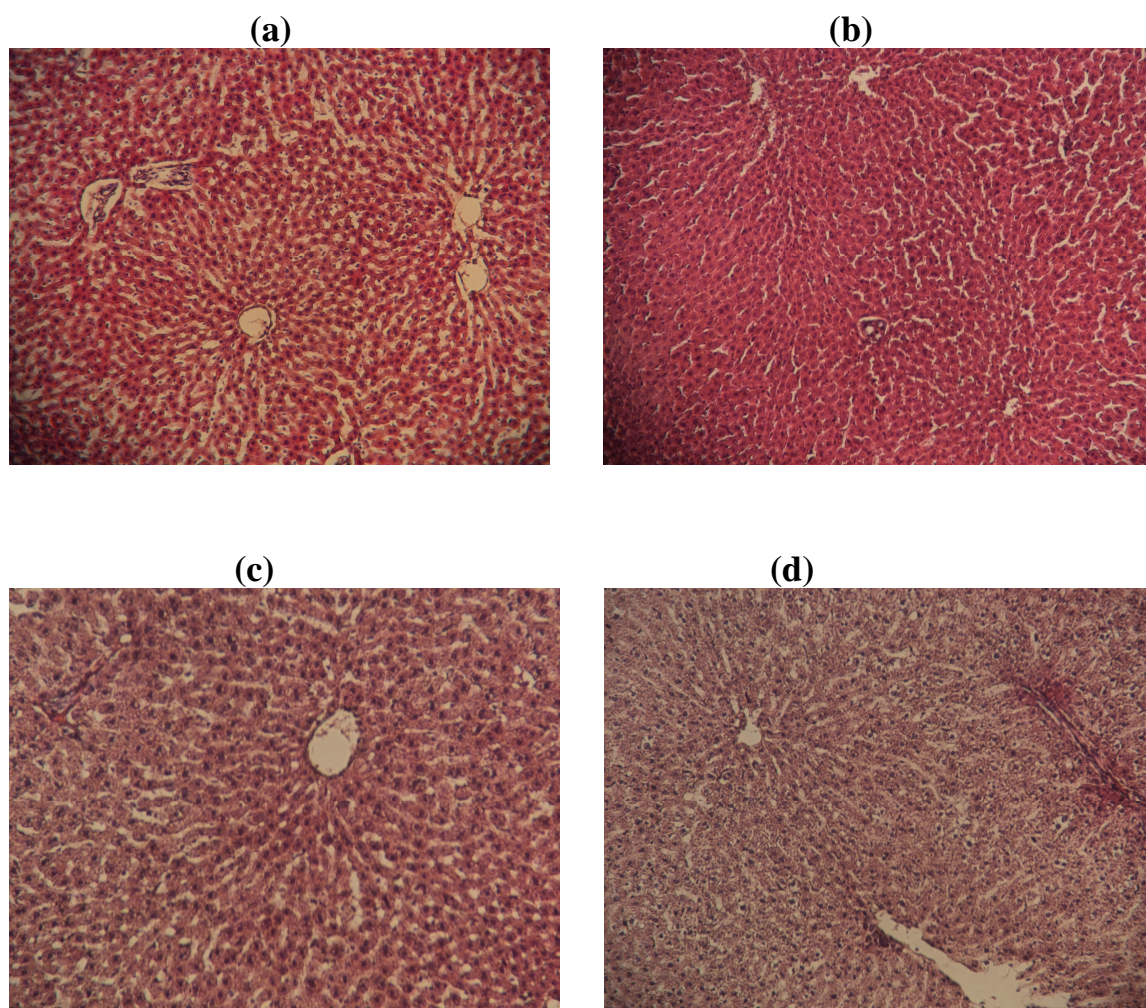


Fig. (14) Histopathological changes in livers of rats treated with ethanol camel milk mixed with camel urine and Silymarin

- (a) = Section of liver cells of group 2 (10% ethanol at 5g/kg), showing centrilobular necrosis and lymphocytic infiltration.
- (b) = Section of liver cells of group 4 treated with mixture of camel milk and urine at "2ml/100gm B. W.", showing almost normal architecture.
- (c) = Section of liver cells of group 5 which treated with mixture of camel milk and urine at "2ml/100gm B. W." + 10% ethanol 5g/kg, showing slight fatty change and lymphocytic infiltration.
- (d) = Section of liver cells of group 3 which treated with Silymarin at 50 mg/kg + 10% ethanol at 5g/kg, showing generalized fatty change.

CHAPTER FOUR

DISCUSSION

The liver is the major organ responsible for the metabolism of drugs and toxic chemicals and therefore, it is the primary target organ for nearly all toxic chemicals (Kaplowitz, 2000; Bissel *et al.*, 2001). Liver damage induced by alcohol (ethanol) is perhaps one of the good studied models of antihepatotoxicity that has been found not only to be useful tools to investigate the pathophysiology underlying the effects of alcohol on the liver but also to evaluate new therapeutic strategies, because acute and chronic alcohol induced liver damage seem to share similar mechanisms (Arteel, 2003).

Alcohol-induced liver damage (ALD) in rodents by intragastric infusion models has been reported to be accompanied by Kupffer cell stimulation as a result of enhanced plasma endotoxin levels (Thurman, 1998). Such mechanisms have been demonstrated to produce steatosis accompanied by inflammatory infiltrates and focal necrosis (Nanji, *et al.*, 1993) and result in the development of fibrosis when extended over a period of months (Tsukamoto, *et al.*, 1986).

Treatment of alcohol-induced liver damage remains limited to supportive measures (Mullen and Dasrathy, 1998). Undoubtedly, the development of effective therapy to prevent or treat ALD will depend on elucidating the underlying mechanisms that contribute to liver injury.

Medicinal usage of plant and/or animal products represents one of the most essential sources of drugs. In Sudan and many other countries camel owners use camel's milk and urine to treat a wide range of health problems. Camel's milk and urine has antibacterial, antifungal, antiviral,

antineoplastic and therapeutic applications on ascetic patients as reported by Amer and Alhendi, (1996) O'haj(1998) and Wisal (2002).

The aims of present study are to make more considerable pharmacological and clinical investigation on camel milk and urine. Our research study data suggest that, there were significant improvement in the observed haematological and serological parameters in rats treated with camel milk and urine.

In this study, the two experiments showed that alcohol (10% ethanol) intake increased the mean values of liver enzymes (AST, ALT, and ALP) when compared to normal rats received normal saline only. These results were in agreement with Rajakrishnan and Menon (2001) who indicated that exposure of hepatocytes to ethanol alters the membrane structure and functions by increasing the leakage of enzymes into the circulation, also, Das *et al.*, (2005) reported that, excess alcohol consumption has been linked with altered liver metabolism and liver damage. In the present study, there was a significant increase in serum metabolites (total proteins, albumin and billirubin) in ethanol group when compared to normal rats received normal saline only. These results were not in line with the results obtained by Ahmed *et al* (2002) who found a decrease in serum total proteins and albumin in ethanol-administered rats and he suggested that, decrease was due to the reduction in the functional ability of liver in ethanol-administered rats. In the current study, serum metabolites (total proteins, albumin and Billirubin) were significantly decreased and maintained near the normal range in all prophylactic groups and also in the treated groups of camel urine alone and the groups treated with camel milk mixed with camel urine.

4.1. Effect of camel urine against ethanol induced liver damage in rats

The present study has been attempted to elucidate, hepatoprotective properties of camel urine in rats. The pharmacological experimental models was carried out to evaluate if the collected early morning camel urine used has the activity that might be considered of interest and to establish explaining the general effects (experimentally) on liver serum enzymes, serum metabolites, haematological parameters and red blood indices values, of normal and intoxicated rats. Such an experimental process represents an essential stage for selecting the most potent hepatoprotective form of camel urine for further detailed pharmacological investigation.

In this study camel urine were tested for its hepatoprotective activity against 10% ethanol (5g/kg) as standard hepatotoxic agent, compared to Silymarin drug (50 mg/kg) as standard anti-hepatotoxic agent.

Different doses of camel urine (1ml/100gm body weight) were tested against liver damage induced by 10% ethanol (5g/kg) and found that it protects the liver against damage with variation in results. The results of the present study of the use of camel urine against alcohol hepatotoxicity in rats indicated a significant hepatoprotective effect. This finding was evidenced by significant reduction of the levels of liver serum enzymes AST, ALT, and ALP, which showed higher levels in ethanol group and these results were correlated with improvement in histopathological picture that appear in ethanol groups as hepatocellular necrosis, apoptosis, fatty accumulation and inflammatory cells infiltration, these results were consistent with the findings of other

authors (Sun *et al.*, 2001, Ali *et al.*, 2001) who found the similar histopathological changes which caused by CCL4 liver toxicity.

Measurement of serum enzyme levels are not enough to evaluate the extent of hepatic injury, however, ALT is considered a liver specific enzyme in rats, but the decrease of enzymes levels points out to a hepatoprotective action of camel urine (Salwa, 2004).

There are few published data concerning camel urine hepatoprotective activity, where as similar hepatoprotective effects were reported by several species of plants such as *Ballanitis agypticaca*, *Rhazya stricta* and *Halophylum tuberculatum* (Ali *et al.*, 2001), *Gymnema sylvestre* and *Curcuma zedoria* (Rana and Avadhood,1992), *Calotropis procera* (Basu *et al.*, 1992). And *Solanum nigrum* (Raja, A. M. 2002), against liver damage.

From the results of current study we noticed that, the levels of serum enzymes significantly reduced when given camel urine before 10% ethanol intoxication and better than the refrence drug (Silymarin). That's may be related to the fact that, camel urine has a potent antioxidant activity and protect against ethanol induced hepatotoxicity.

4.2 Effect of camel milk and urine against ethanol induced liver damage in rats

This experiment was carried out to investigate the protective effects of camel milk and urine against alcohol (10% ethanol) induced damage in the liver of white albino rats. Assessment of harmful effect of alcohol (10% ethanol) and the protective effect of camel milk and urine was based on the analysis of selected parameters, liver serum enzymes,

serum metabolites, haematological parameters and red blood indices values.

Liver damages were assayed by biochemical studies and by three separate serum liver enzymes including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), which are known together as Transaminases, alkaline phosphatase (ALP) which is known as one of cholestatic liver enzymes, all of these enzymes are the reliable indices of liver function. Transaminases (AST and ALT) are important and critical enzymes in the biological processes, therefore, the increase of these enzymes in serum is indicative of liver damage and thus alterations in liver function (Gramiccioni *et al.*, 1996).

In our study, the treatment with camel milk and urine with alcohol (10% ethanol) administration maintained the levels of all investigated parameters (AST, ALT, ALP, total protein, albumen, and billirubin) within the normal values, which indicates improvement in the function of the liver of the treated rat. The decrease in serum activities of liver enzymes AST, ALT, ALP in the rats treated with camel milk and urine before alcohol (10% ethanol) administration could indicate improvement of liver function and protection from the toxicity of alcohol (Khan and Alzohairy, 2011). These findings were confirmed by alleviation of necrosis, apoptosis, fatty accumulation and inflammatory cells infiltration, that clearly observed in ethanol treated group. These findings were consistent with the findings of (Brattin *et al.*, 1985).

The protective effect of camel milk and urine against alcohol induced liver damage in this study could be attributed to its antioxidant activity and it may possibly have chelating effects on toxicants specially alcohol (10% ethanol) (Al-Humaid *et al.*, 2010). It has been reported that camel's milk contain high levels of Vitamins C, A, B2 and E and very

rich in magnesium and other trace elements. These vitamins are antioxidants and found to be useful in preventing the tissues injury caused by toxic agent (Knoess 1979).

Treatment with camel milk and urine could ameliorate alcohol (10% ethanol) induced hepatotoxicity in rats, as demonstrated by the lower serum aminotransferases activities. This effect is in agreement with the commonly accepted view that serum level of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987).

Our results were also in agreement with Kumar *et al.*, (2009) who found that the groups of rats pre-treated with of *Trichosanthes cucumerina* (cucurbitaceae) extract significantly controlled the changes in the biochemical parameters, and exhibited a sharp decrease in the serum enzyme levels. Treatment of rats with camel milk and urine against alcohol intoxication were found to have low levels of serum aminotransferases and alkaline phosphatase after alcohol intoxications. So camel milk and urine consumption has some protective effects and enhance the defense system, these results were in agreement with Cuciureanu *et al.*, (2009) who found that rats, received montelukast sodium before the administration of alcohol (10% ethanol) intoxication, exhibited statistically significant lower levels of AST, ALT, ALP, total proteins, albumin and billirubin as compared to the groups that received alcohol (10% ethanol) only. Similarly, Shen *et al.*, (2009) reported that *Zizyphus jujube* fruit administration prior to alcohol (10% ethanol) intoxication significantly decreased AST and ALT beside attenuation of the histopathology of hepatic injury and ameliorated the oxidative stress in hepatic tissue as compared to the groups of rats that received alcohol only.

CONCLUSION AND RECOMMENDATION

We concluded that the oral administration of alcohol (10% ethanol) (5g/kg) induced damage of liver cells of experimental rats, which was manifested biochemically by significant elevation of liver enzymes AST, ALT, and ALP beside also significant increase in serum metabolites (total proteins, albumin and bilirubin).

On the basis of these results of our prophylactic study we concluded that, camel urine may have an active component(s) which could play an important role as an endogenous antioxidant and/or may act as cyto-protective agent against tissue damage mediated by toxic substances as alcohol (10% ethanol), which better than the reference drug (Silymarin).

In comparison, camel milk mixed with urine shows a significant role in hepatoprotective effects on liver toxicity induced by alcohol (10% ethanol) which better than camel urine alone and the reference drug (Silymarin). Camel milk mixed with urine seems to be a beneficial drink for the prevention of acute and chronic liver toxicity, although further studies are necessary.

*Based on the above mentioned findings we suggest more considerable investigations to be done on the followings:-

- 1- Protective effect of camel milk against alcohol induced liver damage in rats.
- 2- The study of fresh camel colostrum, milk and urine samples during each season is expected to provide evaluation of better different activities.
- 3- Formulation of the active ingredients in the pharmaceutical dosage.

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